SYNTHESIS OF PHOSPHOLIPID ANALOGS

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A Thesis Submitted to the
University of North Carolina at Wilmington in Partial Fulfillment
Of the Requirements for the Degree of
Master of Science

Department of Chemistry

University of North Carolina at Wilmington

2003

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This thesis has been prepared in the style and format

consistent with the journal

Journal of the American Chemical Society

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ABSTRACT

Phospholipids are fundamental components of all biological membranes. They form the lipid bilayer of the membrane, and their structure is a major determinant in the structure and function of cell membranes. Two synthetic pathways to phospholipid analogs are explored in this work. First, a synthetic pathway starting from glycidol to ultraviolet active diacylglycerides (DAGs) that that have *sn*-1 and *sn*-2 ester-linked fatty acids and a chromophore at *sn*-3 was developed. These DAG derivatives could serve as standards to elucidate the structures of natural phospholipid derivatives. Second, a synthetic pathway to a tethered phospholipid analog with a fluorophore anchored between the *sn*-1 alkyl chains was developed. This type of lipid analog could serve as a probe to study molecular interactions within the membrane.

ACKNOWLEDGEMENTS

First, I would like to acknowledge my advisor, Dr. Pam Seaton, for giving me this project, teaching me so much along the way, and for always being patient and supportive. I would also like to thank my committee members, Dr. Paulo Almeida and Dr. Ned Martin. Thanks to Dr. Almeida for introducing me to some very interesting concepts of biochemistry, and thanks to Dr. Martin for always having work for me to do and for being incredibly patient.

I would also like to acknowledge my family who has always been encouraging and supportive. Thanks to my son, Kyler, and his dad, Jon, for being so wonderful when I have been so busy. You guys have been really patient with me while I have been in school, and I really appreciate all of your support and sacrifices. Also, thanks to my mom who has always worked incredibly hard to take care of her family and has always supported me in my choices- some good and some not so good. Thanks to my brother, Justin, for being one of my best friends through some pretty interesting times. Thanks to my Granny Rae for being supportive in her own, special way.

In the past six years I have made two of the best friends that I have ever had; so, finally, I would like to thank my two best friends, Melissa Dominguez and Jennifer Jones. The best thing about you two is how different you are, and I really appreciate you both for who each of you are and how great you guys are in your own ways. Thanks to Melissa for showing me that it is important to relax once in a while, preferably by playing lots of pool and a little foosball. And, thanks to Jennifer for introducing me to the concept of organization and for endless rides, lunches and long phone calls.

DEDICATION

I would like to dedicate all of my work to my son, Kyler, who motivates me to always do my best at anything that I try. I hope that I will be a good role model for him and inspire him in the same way.

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INTRODUCTION

Phospholipids are fundamental components of all biological membranes. They form the lipid bilayer of the membrane, and their structure is a major determinant in the structure and function of cell membranes. There are two major classes of phospholipids, glycerophospholipids and sphingophospholipids. Glycerophospholipids, the focus of this research, consist of a glycerol backbone, two ester-linked fatty acid chains in the *sn*-1 and *sn*-2 positions, and a substituted phosphate attached by phospho-ester linkage in the *sn*-3 position (Figure 1).[1] It is important to note that there are numerous individual phospholipid species. For example, the *sn*-1 and *sn*-2 fatty acid composition may vary in length and degree of unsaturation, and the substitution of the phosphate group at *sn*-3 may also vary. Variations in phospholipid composition of the membrane can affect both the structure and function of the membrane, thereby influencing some cellular processes.[1,2] Because of their influence on membrane structure and function, it is important to be able to study lipid composition and interactions in the membrane.

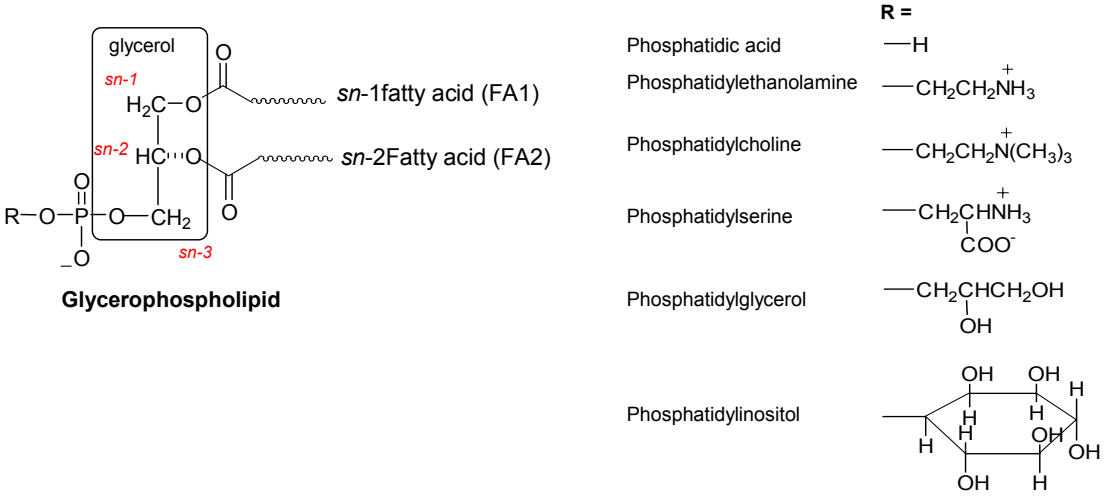


Figure 1: Common glycerophospholipid structures.

A synthetic route to phospholipid analogs would make it possible to both elucidate the structures of individual natural phospholipid species and provide a probe to study phospholipid interactions in the membrane. The syntheses of two types of phospholipid analogs are explored in this work. Synthesis of standard glycerophospholipid analogs resembling the structures in Figure 1 is depicted in Figure 2. Synthesis of a tethered phospholipid analog that would span the entire lipid bilayer is shown in Figure 3.

For the initial synthesis, choice of starting materials was important. Glycidol (**1**) is used to provide the glycerol backbone of the lipid. This was chosen because the epoxide can be opened and selectively substituted at the primary carbon. Selective substitution of the primary carbon is necessary in order to selectively add a specific *sn*-1 fatty acid. The S(-)-glycidol isomer (**1a**) must be used so that the primary epoxide carbon becomes the *sn*-1 position of the resulting lipid.[1,3] The p-methoxybenzyl chloride (**2**) group was chosen as the *sn*-3 protecting group because it is a good chromophore that is easily attached, and is reported to be easily removed under relatively mild conditions.[4]

After protection of the glycidol primary alcohol with the p-methoxybenzyl chloride (**2**), the epoxide is opened by nucleophilic addition at the primary epoxide carbon by the carboxylate ion of the *sn*-1 fatty acid (**4**) to give the monoacylglyceride.[5] The resulting secondary hydroxyl group reacts with the acid chloride of the *sn*-2 fatty acid (**6**) to form the second ester linkage of the diacylglyceride (**7**).[6] The final step for the synthesis of phospholipids would be to remove the *sn*-3 protecting group and replace it with the desired substituted phosphate group to yield the glycerophospholipid analog (**8**).

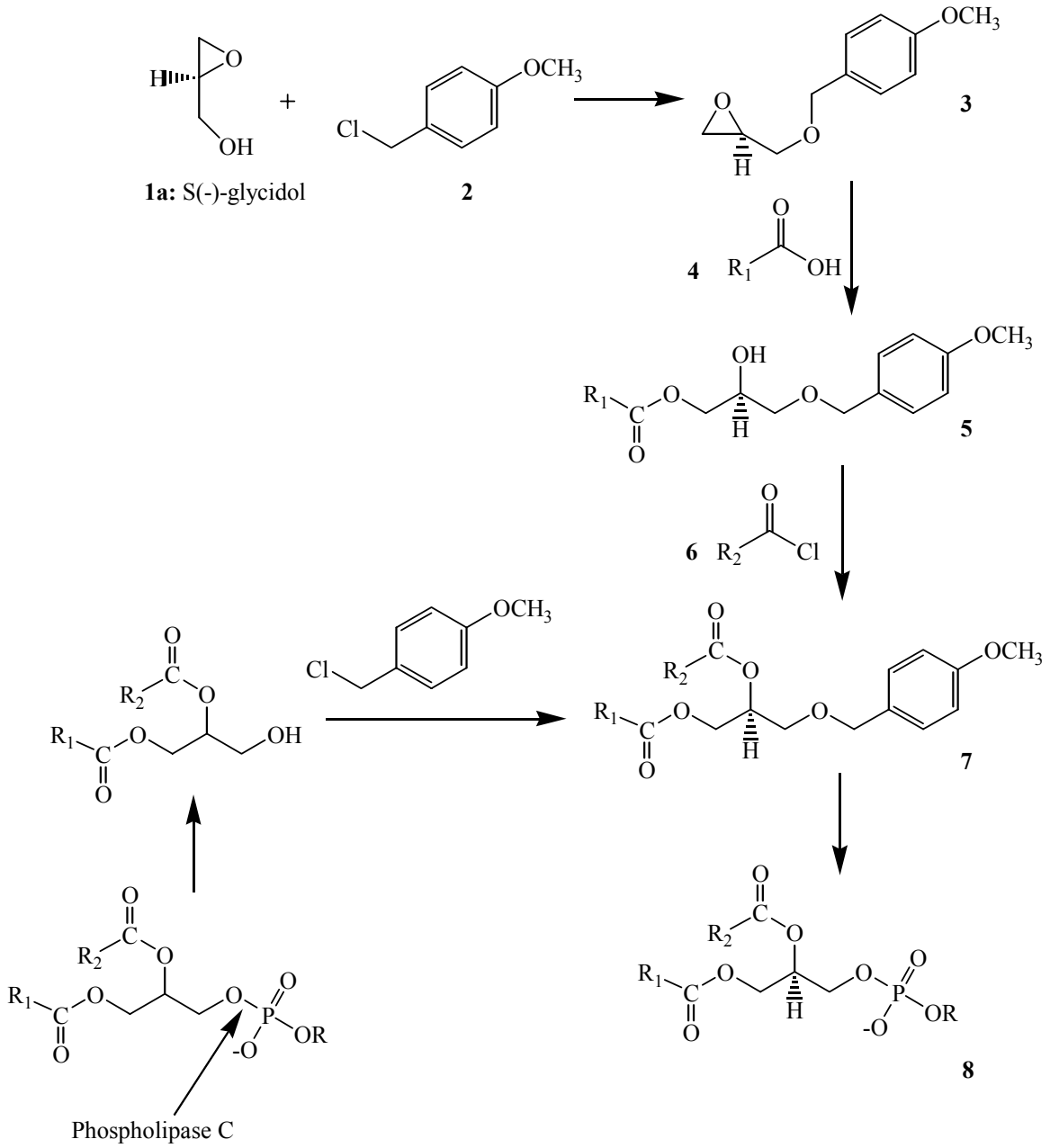


Figure 2: Proposed route to glycerophospholipid analogues, and the hydrolysis of a glycerophospholipid, using phospholipase C, followed by derivatization of the resulting diacylglycerol with selected chromophore.

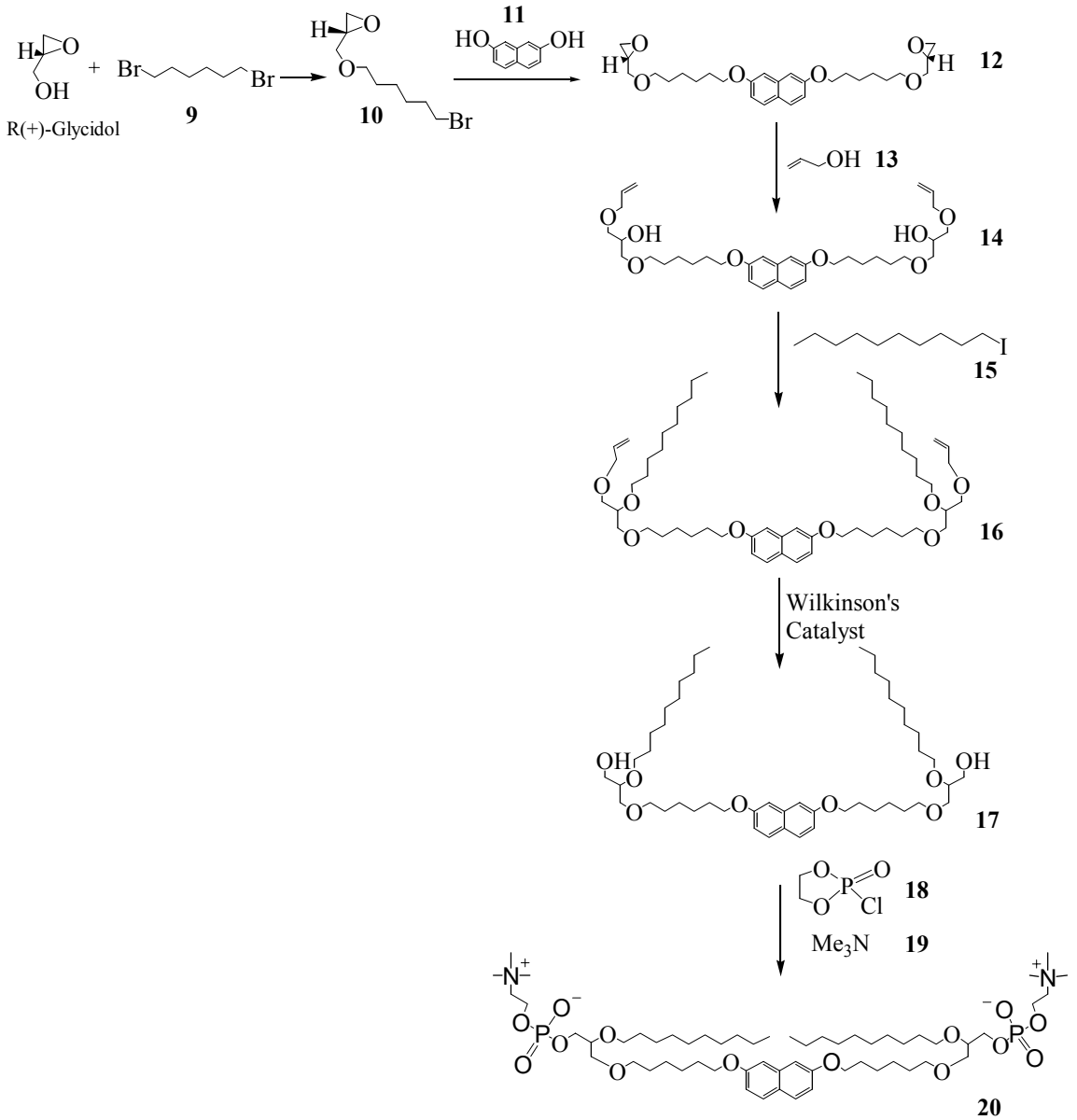
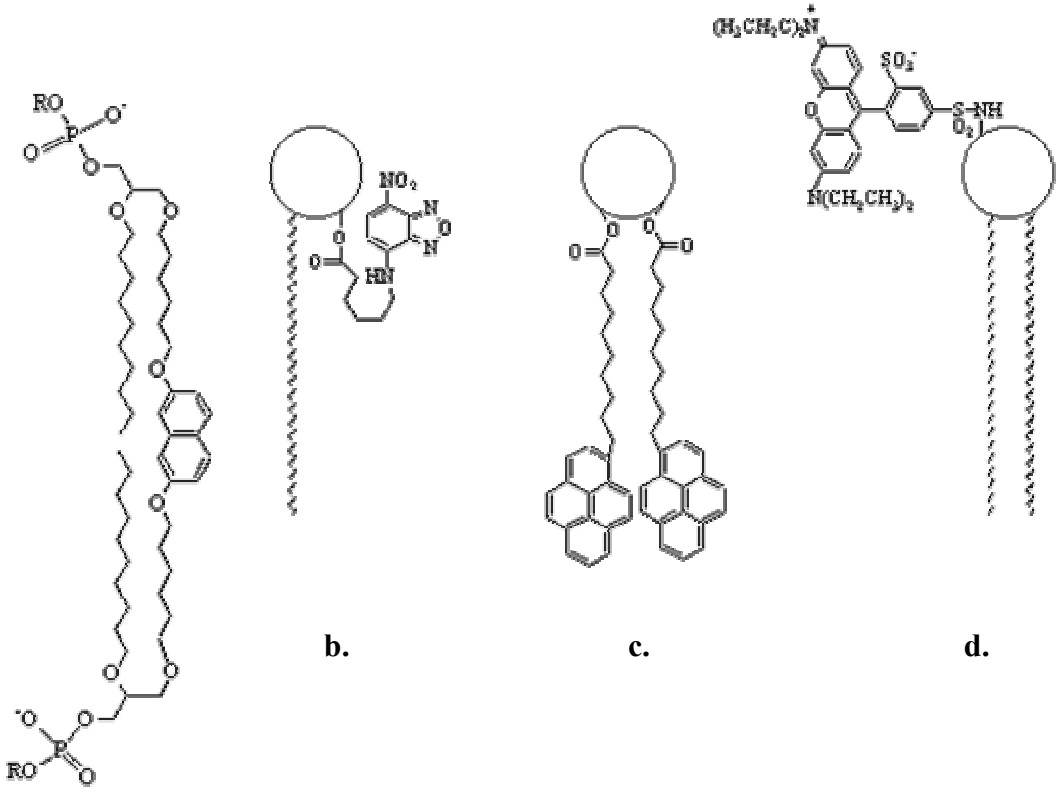


Figure 3: Proposed synthesis of tethered phospholipid.

A second synthetic route, which would yield phospholipid analogs that span the bilayer was also explored.[7,8] By linking the *sn*-1 fatty acids of two individual lipids using a fluorophore, lipid structures of this type could serve as molecular probes.[9] A variety of membrane probes are available. Among these probes are lipid analogs with fluorophores incorporated into the fatty acid chains (Figure 4b, c) or the polar head group (Figure 4d).[9] However, for the structure proposed in this work (Figure 4a), the fluorophore is held between the terminal carbons of the *sn*-1 fatty acids, thereby forcing it to remain in the membrane, anchored by the polar head group on either end.



a. **b.** **c.** **d.**

Figure 4: Phospholipids with fluorophores incorporated into their structure. a) Structure proposed in this research. Structures b-d depict available lipid analogs with fluorophores incorporated into the fatty acid chains (b, c) or the polar head group (d).[9]

Ensuring that the fluorophore is in the hydrophobic part of the membrane would make it possible to study peptide interactions with the membrane (Figure 5). For instance, one could determine if a peptide was at the surface of the membrane (Figure 5a) or if it passed through the hydrophobic region of the membrane (Figure 5b) using fluorescence resonance energy transfer.[9] This would require that either the emission or excitation spectrum of the fluorophore in the lipid overlapped the excitation or emission spectrum of the peptide, respectively. If the peptide passed through the membrane, a nonradiative transfer of energy would occur resulting in a change in fluorescence intensity. However, if the peptide remained at the surface of the membrane, no change in fluorescence would be observed.

a.

No change in fluorescence intensity

b.

Decrease in fluorescence intensity due to nonradiative transfer of energy

Figure 5: Lipid bilayer with fluorescent lipid probe and fluorescent peptide either at the membrane surface (5a) or in the hydrophobic region of the membrane (5b).

The proposed route to this type of phospholipid analog is shown in Figure 3. Again, glycidol is used because of the selectivity for substitution at the primary epoxide carbon. However, for this process R(+)-glycidol (**1b**) is used because the primary

epoxide carbon will become the *sn*-3 position of the resulting lipid analog; whereas, it was the *sn*-1 position in the previous synthesis. The *sn*-1 carbon chain is first attached to carbon 3 of the glycidol to give **10**. Two molecules of **10** are attached through ether linkages to 2,7-dihydroxynahpthalene (**11**) to give the tethered species (**12**). In this synthesis ether linkages were chosen because they offer more stability than esters and they will prevent confusion due to transesterification. The epoxide is opened with allyl alcohol (**13**), which will provide a protecting group at the *sn*-3 position of **14**.[8] The *sn*-2 substituent is attached to both secondary alcohols of **14** through ether linkages. Removal of the allyl protecting group from **16** would allow for attachment of the desired phosphphosphatidyl choline to give **20**.

RESULTS AND DISCUSSION

Synthesis of Glycerophospholipid Analogs

The initial goal of this research was to develop a synthetic pathway to glycerophospholipid analogs (Figure 2). The proposed synthetic route incorporates a UV-active diacylglyceride derivative, with the chromophore at *sn*-3, to form a glycerophospholipid analog. The first step in this synthesis is protection of the glycidol alcohol, which will become the *sn*-3 position of the lipid (Figure 6). Racemic glycidol was used for the development of this synthesis; S(-)-glycidol would be incorporated to make glycerophospholipid analogs.[3]

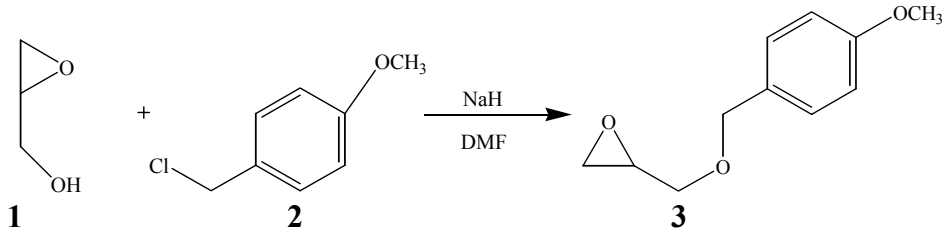


Figure 6: Synthesis of **3**.

The p-methoxybenzyl moiety was chosen as the protecting group for several reasons. First, it was relatively easy to attach to the glycidol alcohol. NaH deprotonated the alcohol, and the resulting alkoxide was reacted with p-methoxybenzyl chloride to form the p-methoxybenzyl glycidol ether (**3**) through an S_N2 mechanism.[4] The methoxybenzyl group is reported to be stable under the conditions required to add the *sn*-1 and *sn*-2 fatty acids, and it is reported to be more easily removed under mild oxidative conditions than an unsubstituted benzyl group, which requires a strong acid or reductive conditions.[4] Additionally, the p-methoxybenzyl group will provide a chromophore for the series of compounds produced in this scheme. This will make the compounds easily detected by UV and result in a UV-active diacylglyceride derivative after the addition of the *sn*-1 and *sn*-2 fatty acids.

The structure of the p-methoxybenzyl glycidol ether (**3**) was confirmed by ^1H NMR, ^{13}C NMR, and two-dimensional NMR experiments. $^1H^1H$ COSY (Figure 7) was used to confirm proton assignments, and HMQC was used to assign carbon signals; NMR assignments are given in Table 1. Because the glycidol protons appear as five separate 1H signals, $^1H^1H$ COSY was integral in assigning proton signals for this structure. The multiplet at 3.2 ppm (the *sn*-2 H) couples to the two doublets of doublets at 2.6 ppm and 2.8 ppm as well as the two doublets of doublets at 3.4 and 3.7 ppm. The signals at 2.6 and 2.8 ppm were assigned as the primary epoxide protons, and the signals at 3.4 and 3.7 ppm were assigned as the diastereotopic *sn*-3 protons. These assignments were made because protons that are attached to sp^3 carbons of a strained ring, such as the primary epoxide protons, typically have a lower chemical shift than protons attached to sp^3 carbons that are not part of a ring, such as the *sn*-3 protons.[10] The benzyl protons are at

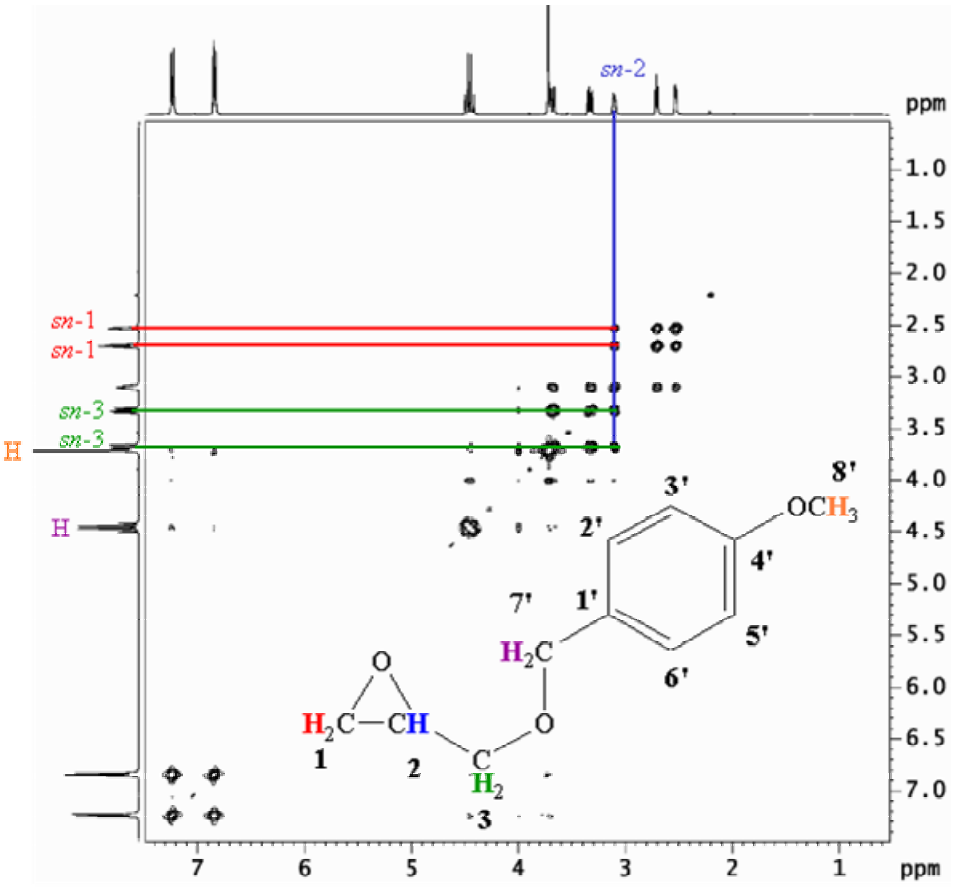


Figure 7: $^{1}H^{1}H$ COSY of **3**.

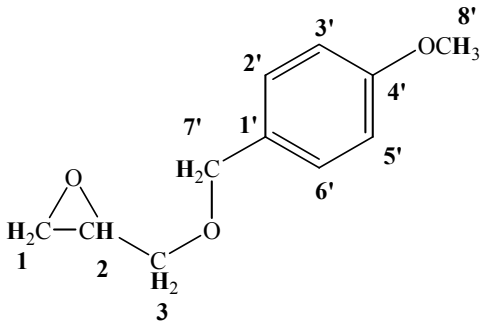
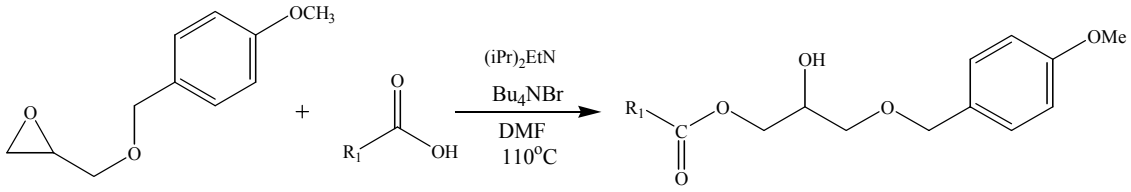


Table 1: NMR data for **3** in $CDCl_3$

	^{1}H δ (ppm)	Integration	Multiplicity	J (Hz)	^{13}C δ (ppm)
1	2.5	1 H	dd	2.7, 5.0	44
	2.8	1 H	dd	-	-
2	3.2	1 H	m	-	51
3	3.4	1 H	dd	6.0, 11.4	71
	3.7	1 H	dd	11.4, 2.9	-
1'	-	-	-	-	130
2',6'	7.3	2 H	d	8.6	129
3',5'	6.9	2 H	d	8.6	114
4'	-	-	-	-	158
7'	4.5	2 H	dd	23.3, 11.6	73
8'	3.9	3 H	s	-	56

4.5 ppm, the methoxy protons appear as a 3H singlet at 3.8 ppm, and the para substituted benzene ring shows two 2H doublets at 6.9 and 7.3 ppm.

The next step in this synthesis was to selectively add the first fatty acid to the primary epoxide carbon (Figure 8). For this work, two saturated fatty acids, palmitic acid (C16:0) and stearic acid (C18:0), were used as the *sn*-1 fatty acid. These are commonly found as *sn*-1 fatty acids in naturally occurring lipids.[1] Diisopropylamine was used to deprotonate the carboxylic acid, and the resulting carboxylate anion selectively attacked at the primary epoxide carbon, resulting in an ester linked fatty acid at the *sn*-1 position, leaving the free secondary alcohol (**5a,b**). Tetrabutylammonium bromide, a phase transfer catalyst was also found to be necessary in this reaction.[5] Although there are not two phases in this reaction, the reaction does not proceed without the phase transfer catalyst. It is possible that the phase transfer catalyst prevents micelle formation of the deprotonated fatty acids.



3 **4a**: palmitic acid **4b**: stearic acid **5a**: *sn*-1 palmitic acid **5b**:*sn*-1 stearic acid

Figure 8: Synthesis of **5 a-b**.

^{1}H NMR (Figure 9) was used to prove that p-methoxybenzylglycidol (**3**) had been converted to the *sn*-1 acyl derivative (**5a-b**). NMR data for **5a** and **5b** were identical except for the integration of the fatty acid alkyl chain protons. The addition of a fatty acid is apparent by the appearance of fatty acid alkyl chain protons, 0.5 ppm through 2.5 ppm, and integration is consistent with the addition of one fatty acid. The signal for the

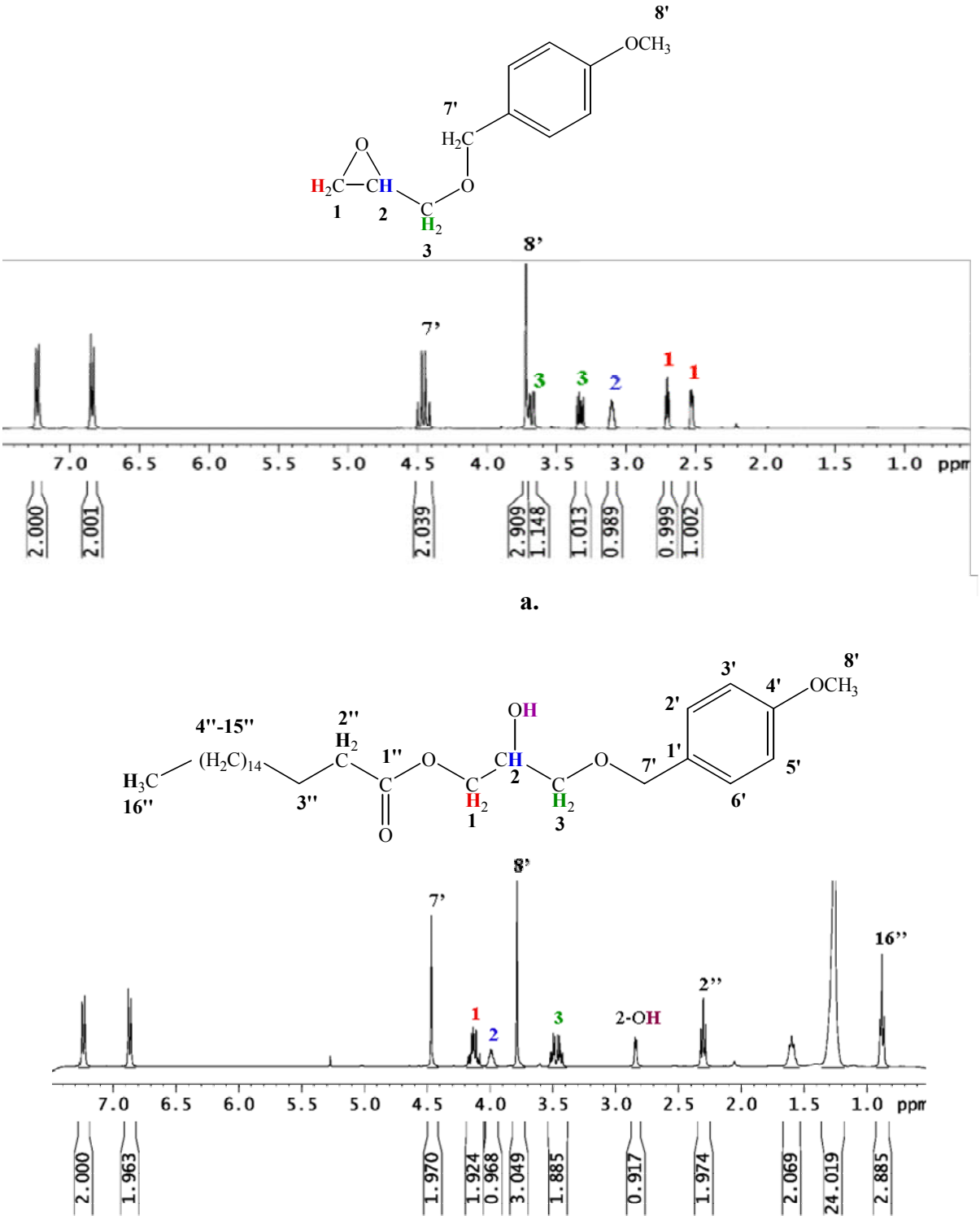


Figure 9: ^{1}H NMR of **3** (a) and **5** (b).

protons alpha to the carbonyl (2.1 ppm) integrates for 2H, and the terminal fatty acid methyl group is a 3H triplet at 0.7 ppm.

The placement of the fatty acid at position 1 is confirmed by the shifting of the *sn*-1 protons from 2.6 and 2.8 ppm to 4.3 ppm, which is a typical of protons at acylated carbons.[10] The *sn*-2 proton also shifted, from 3.2 to 4.0 ppm, due to the opening of the epoxide ring. A new 1H doublet at 2.8 ppm was assigned as the *sn*-2 OH. After addition of D_2O to the NMR sample, this signal at 2.8 ppm disappeared, verifying that this is an exchangeable alcohol proton (Figure 10). The benzyl protons are still a 2H signal at 4.5 ppm, but after the epoxide opens, this signal is a singlet.

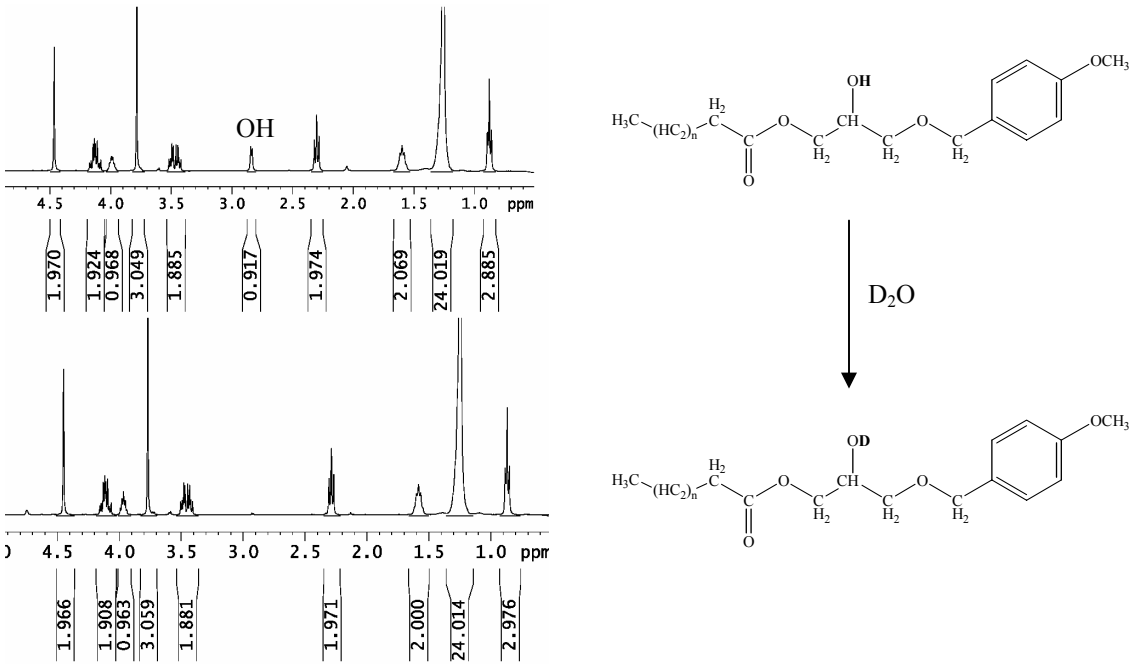


Figure 10: ^{1}H NMR spectra of **5** before (top) and after (bottom) addition of D_2O.

The $^{1}H^{1}H$ COSY of this compound (Figure 11) was also used to help assign the glycidol protons and confirm the placement of the first fatty acid. The 1H multiplet at 4.0 ppm is assigned as the *sn*-2 protons because it couples the *sn*-1 protons at 4.3 ppm and the *sn*-3 protons at 3.4 ppm. This signal also couples to the alcohol proton (2.8 ppm), again verifying that the alcohol is at *sn*-2 and the fatty acid must be at *sn*-1. HMQC was used to assign carbon chemical shifts; NMR data is given in Table 2.

Oleic acid (C18:1) was chosen as the *sn*-2 fatty acid because it is commonly found in natural lipids (Figure 12).[1] Also, it has one double bond, making it easier to determine that one oleic acid has been added. ^{1}H NMR will show the appearance of two double bond protons when one oleic acid has been added to the monoacyl derivative, **5**.

The addition of the second fatty acid to the *sn*-2 alcohol occurs by first converting the fatty acid to its corresponding acid chloride (**6b**), which is then reacted with the secondary alcohol of **5**. This results in an ester-linked *sn*-2 fatty acid.[6] This reaction produces HCl, so an excess of triethylamine is used to neutralize the HCl, to prevent breakdown of the remainder of the molecule.

Addition of oleic acid to the *sn*-2 position is confirmed by ^{1}H NMR (Figure 13). As previously mentioned, the addition of oleic acid is confirmed by the appearance of a 2H multiplet at 5.4 ppm that corresponds to the two double bond protons of oleic acid. Also, the *sn*-2 glycerol proton shifts downfield to from 4.0 to 5.2 ppm, typical of shifts of an acylated methine. Additionally, the *sn*-2 OH doublet at 2.8 ppm disappears with the addition of the fatty acid. Signals from the benzyl protons (4.5 ppm), methoxy protons (3.7 ppm), and aromatic protons (6.8 ppm and 7.3 ppm) remain virtually unchanged. HMQC was used to assign carbon signals; NMR data is given in Table 3.

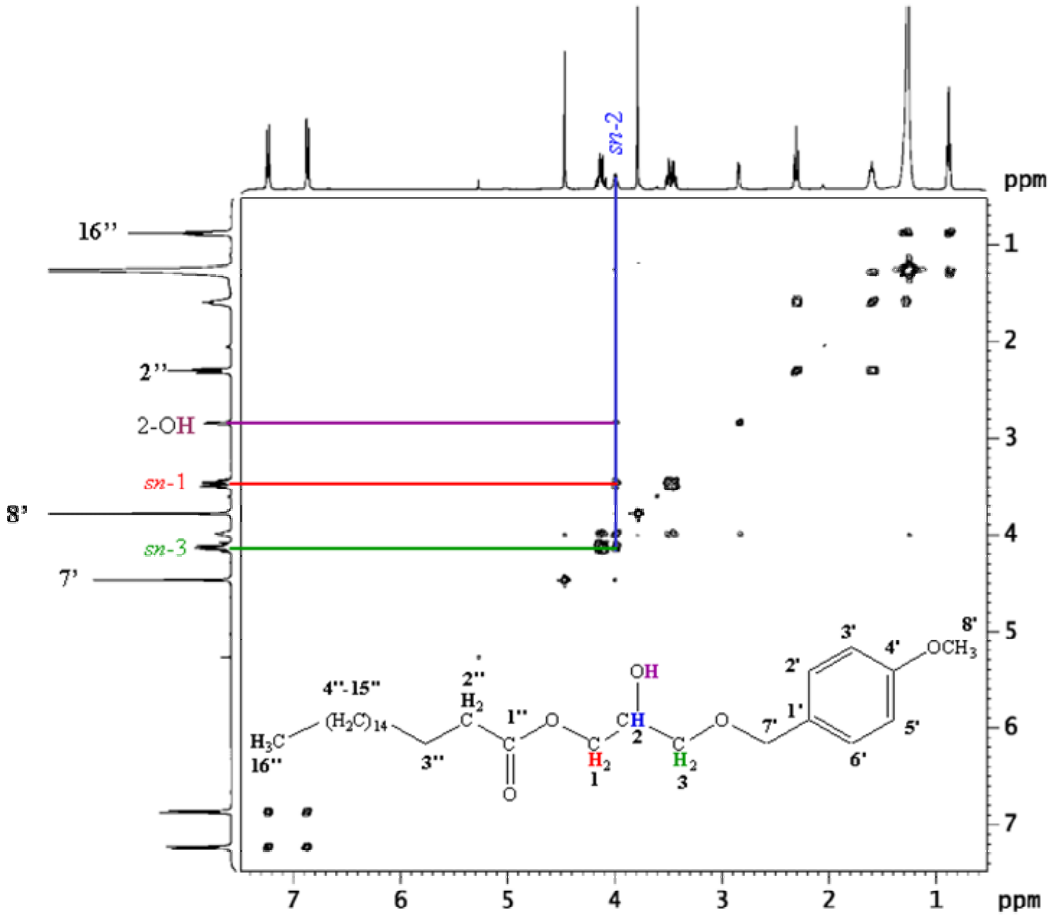


Figure 11: $^{1}H^{1}H$ COSY of **5**.

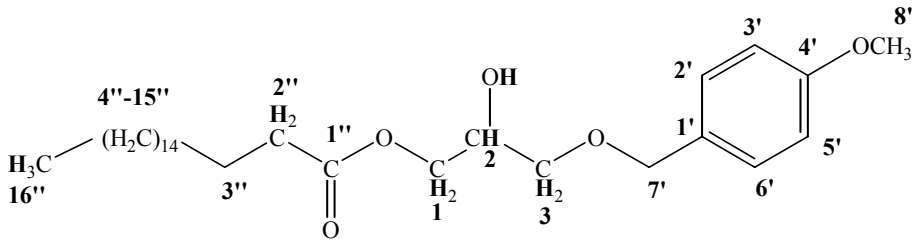
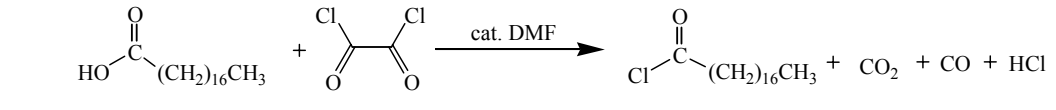


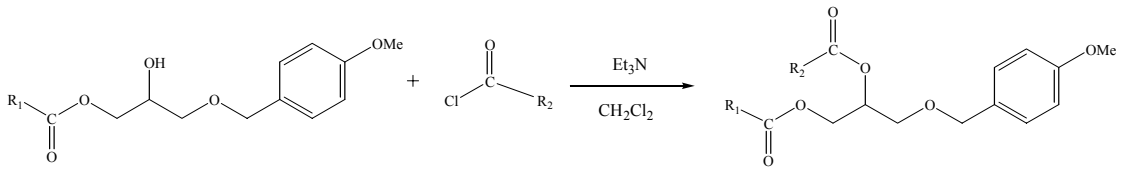
Table 2: NMR data for **5** in $CDCl_3$

	^{1}H δ (ppm)	Integration	Multiplicity	J (Hz)	^{13}C δ (ppm)
1	4.1	2H	m	-	65.3
2	3.9	1H	m	-	68.8
2-OH	2.8	1H	d	4.6	-
3	3.4	2H	m	-	70.6
1'	-	-	-	-	129.7
2',6'	7.2	2H	d	8.6	129.3
3',5'	6.9	2H	d	8.6	113.8
4'	-	-	-	-	159.3
7'	4.5	2H	s	-	73
8'	3.8	3H	s		55.1
1"	-	-	-	-	173.8
2"	2.3	2H	t		34
3"	1.6	2H	-		24.8
4"-15''	1.2	24H	m		*
16"	0.9	3H	t		14

*Carbon signals ranging from 22.6 - 31.8: 22.6, 29.1, 29.2, 29.3, 29.4, 29.5, 29.6, 29.6, 31.8



6 **6b**

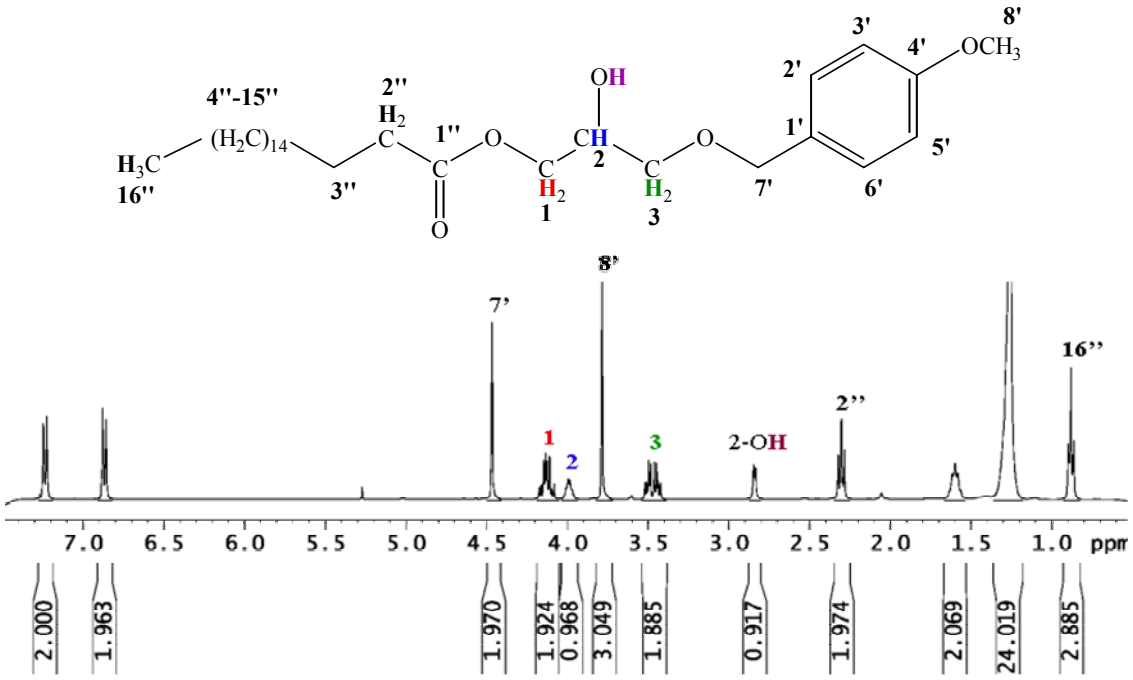


5a: *sn*-1 palmitic acid
5b: *sn*-1 stearic acid

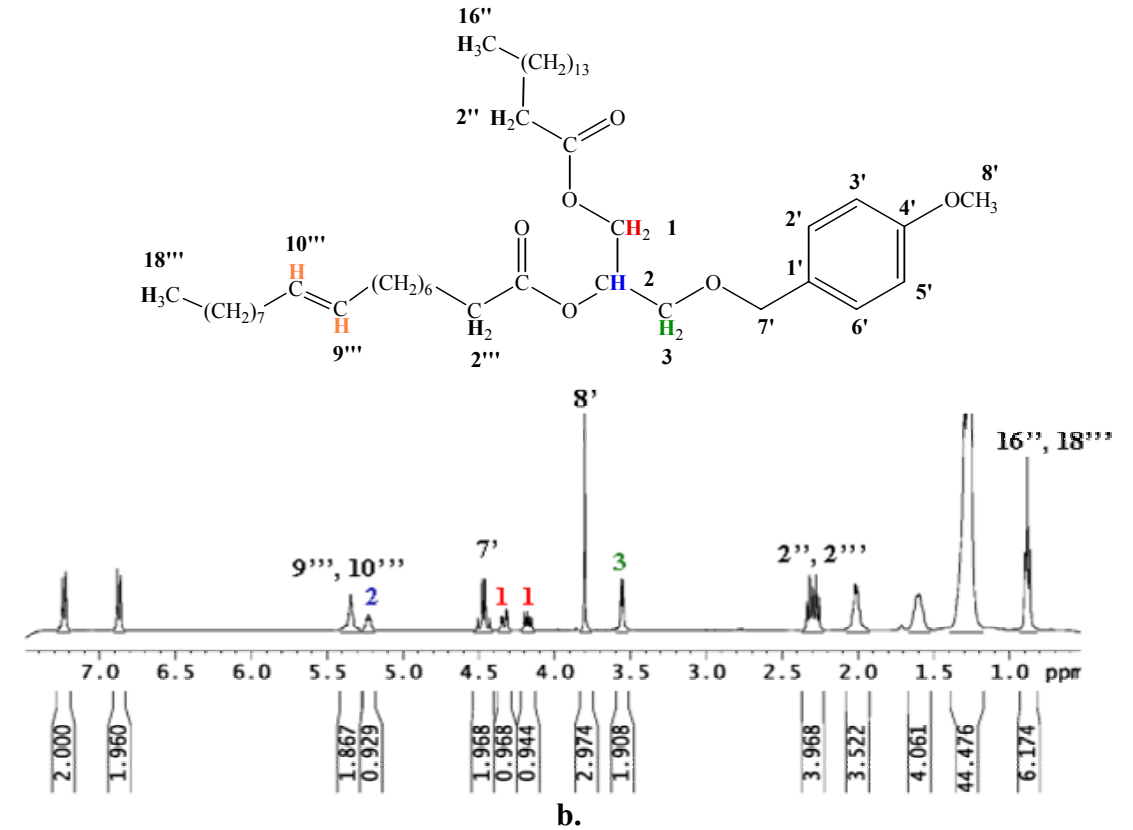
6b

7a: *sn*-1 palmitic, *sn*-2 oleic
7**b**: *sn*-1 stearic, *sn*-2 oleic

Figure 12: Synthesis of **7 a-b** by addition of oleic acid chloride, **6b**, to **5 a-b**.



a.



b.

Figure 13: ^{1}H NMR of **5** (a) and **7**(b).

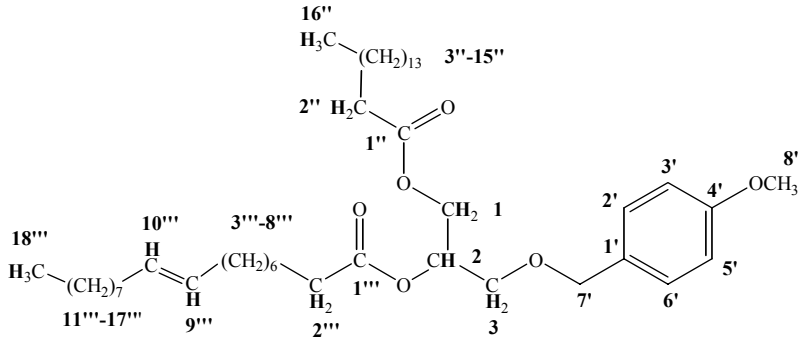


Table 3: NMR data for **7a** obtained in $CDCl_3$.

	^{1}H δ (ppm)	Integration	Multiplicity	J (Hz)	^{13}C δ (ppm)
1	4.15	1H	m	-	62.6
	4.35	1H	m	-	-
2	5.2	1H	m	-	70.0
3	3.55	2H	d	5.2	67.8
1'	-	-	-	-	129.9
2', 6'	7.25	2H	d	8.5	129.2
3', 5'	6.85	2H	d	8.6	113.7
4'	-	-	-	-	159.0
7'	4.45	2H	m	-	72.9
8'	3.79	3H	s	-	55.2
1"	-	-	-	-	173.5
2"	2.29*	2H	m	-	34.5
3"	1.60*	2H	m	-	**
4"-15"	1.27*	24H	m	-	**
16"	0.88*	3H	t	6.4	14.1
1'''	-	-	-	-	174.0
2'''	2.29*	2H	m	-	34.9
3'''	1.60*	2H	m	-	**
4'''-7'''	1.27*	8H	m	-	**
8'''	2.00*	2H	m	-	**
9'''	5.34*	1H	m	-	129.6
10'''	5.34*	1H	m	-	129.6
11'''	2.00*	2H	m	-	**
12'''-17'''	1.27*	12H	m	-	**
18'''	0.88*	3H	t	6.4	14.1

*Overlapping signals: 2" and 2'''; 3" and 3'''; 4"-15",4'''-7''', and 12'''-17'''; 8''' and 11'''; 9''' and 10'''; and 16" and 18"

**Carbon signals ranging from 22.6 to 31.9 ppm: 22.6, 29.1, 29.2, 29.3, 29.3, 29.4, 29.6, 29.6, 29.7, 29.7, 31.9

Compounds **7a** and **7b** can now serve as UV active diacylglyceride (DAG) derivatives. A series of these compounds with varying fatty acids could be formed and used as standards for identifying naturally occurring phospholipids. Treatment of natural phospholipids with phospholipase C would give an *sn*-1,2 diacylglycerol that could be reacted with p-methoxybenzyl chloride to yield derivatized lipids for comparison to the synthetic standards (Figure 2).[1,4]

The final step for this synthesis of phospholipids was to remove the methoxybenzyl protecting group and replace it with the desired phosphate group. Two methods of removing the protecting group were attempted (Figure 14). First, the DAG derivative (**7**) was treated with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) to remove the methoxybenzyl group by oxidation.[11] The second method, another oxidation, was a solid phase reaction where compound **7** was applied to clay supported ceric ammonium nitrate (CAN) and subjected to microwave radiation.[12] The esters at *sn*-1 and *sn*-2 positions are reported to be stable to conditions required for either reaction.[11,12]

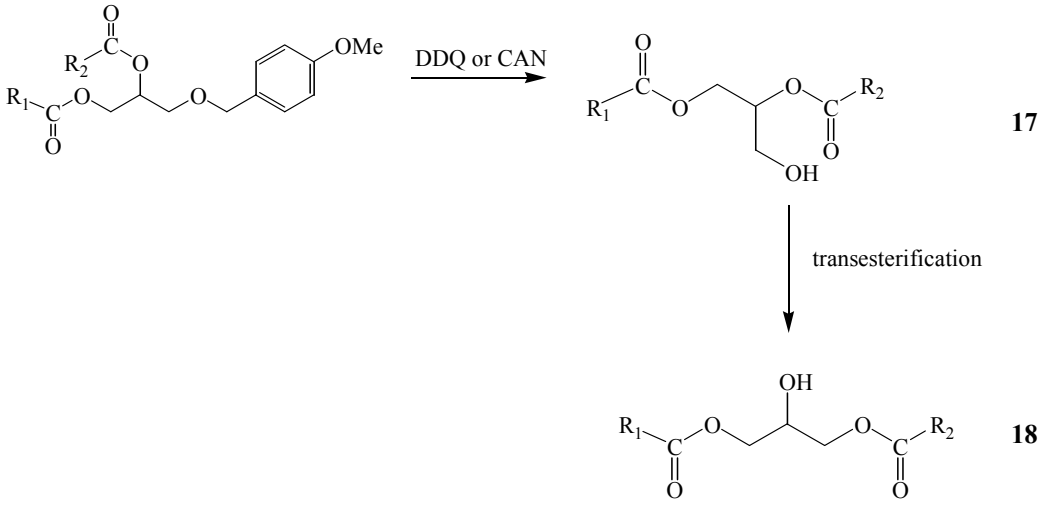


Figure 14: Removal of p-methoxybenzyl group followed by transesterification.

Both reactions (DDQ and CAN) used to remove the methoxybenzyl group resulted in a mixture of diacylglycerides, which is probably due to transesterification of the *sn*-2 fatty acid to the *sn*-3 position once the *sn*-3 protecting group is removed (Figure 14). The DAGs produced in these reactions could not be resolved through flash column chromatography (FCC). In the ^{1}H NMR spectrum of the DAG mixture (Figure 15), the signal for the fatty acid protons alpha to the carbonyl (2.35 ppm) integrated for 4H, accounting for two fatty acids. The *sn*-2 OH signal, seen previously in Figures 9-11, reappears in this spectrum as a 1H doublet at 2.4 ppm. Another indication that this is mainly *sn*-1,3 diacylglycerols is the chemical shift range for the five glycidol protons, 4.0 to 4.5 ppm. In previous spectra, it was shown that the *sn*-1 glycidol proton signal was at 4.3 ppm when there was an ester at that position, and the *sn*-2 proton signal was at 5.2 ppm when an ester was at that position and at 4.0 when a free alcohol was present. The five proton signal between 4.0 and 4.5 ppm accounts for *sn*-1 and *sn*-3 glycidol protons with ester-linked fatty acids at each position and one *sn*-2 glycidol proton with an alcohol at that position. These results indicate that the *sn*-2 oleic acid has transesterified to the *sn*-3 position; however, the olefinic proton signal at 5.4 ppm does not integrate for 2H, indicating that, while there are two fatty acids per molecule, there is not necessarily one palmitic acid and one oleic acid. The major DAGs are the result of both intermolecular and intramolecular transesterfication, so that they may have any combination of oleic and palmitic acids attached. Both the DDQ and CAN gave similar results. In order to attach a phosphate group to the sn-3 position, a new protecting group that could be removed under conditions that would minimize transesterification should be considered.

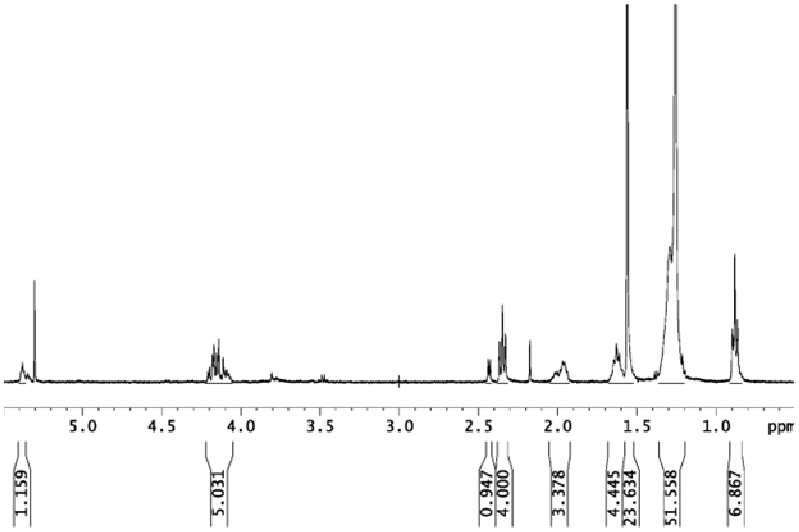


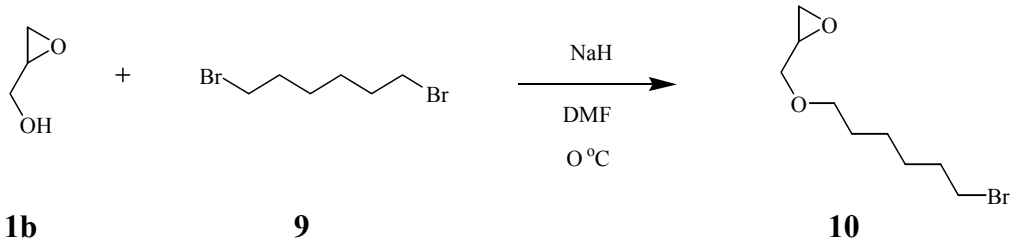
Figure 15: ^{1}H NMR of the product after removal of p-methoxybenzyl group.

Synthesis of Phospholipid Probes

The second synthetic route explored in this research (Figure 3) was to create a phospholipid analog that spans the entire bilayer. This phospholipid analog has ether linked chains at each position on the glycerol backbone to provide stability and avoid transesterification problems associated with the esters in the previous synthesis. The lipid analog created by this synthesis has a fluorophore incorporated into the membrane-spanning carbon chain. This structure should ensure that the fluorophore remains in the hydrophobic part of the membrane, anchored on either end by the polar head group. This would be relevant in determining if peptides were associated with the membrane surface, or if they pass through the hydrophobic region. Studies like this would incorporate fluorescence resonance energy transfer, and therefore require that the fluorophore used in the lipid analog has an excitation or emission spectrum that overlaps the emission or excitation spectrum of the peptide in question, respectively (Figure 5).[9] 2,7-Dihydroxynaphthalene was used for the development of this synthetic route because it is

commercially available and relatively inexpensive. However, once the synthetic method is developed, this could be replaced with a fluorophore that has the desired spectral properties and similar reactivity.

Racemic glycidol (**1**) was used as the starting material as in the previous synthesis for development of reaction conditions. However, R(+)-glycidol (**1b**) was used after initial optimization because the primary epoxide carbon of glycidol will become the *sn*-3 position, whereas it was the *sn*-1 position in the previous synthesis. There were no observed spectral differences between the compounds made with racemic glycidol and those made with R(+)-glycidol. The first step was to begin the formation of the bilayer-spanning chain with the fluorophore incorporated. NaH was used to deprotonate the glycidol alcohol (**1**) which then reacted with 1,6-dibromohexane (**9**) to form 1-bromohexyl glycidol ether (**10**) through through an S_N2 mechanism (Figure 16). A large excess of the dibromohexane was used in order to prevent any side reactions of the deprotonated glycidol with other glycidol molecules and to prevent linking two molecules of glycidol (**1**) with the dibromohexane (**9**). At this point, the compound cannot be detected by UV, so it was necessary to analyze reaction progress and product purity by staining TLC plates with p-anisaldehyde and by ^{1}H NMR. The product was purified by FCC, and the purity of fractions was confirmed by GC/MS and NMR. The M^+ and $M+2^+$ values are 237 and 239.



1b **9** **10**

Figure16: Synthesis of **10**.

NMR experiments were used to confirm the structure of this compound (**10**). $^{1}H^{1}H$ COSY was used to assign proton signals (Figure 17), and HMQC was used to assign carbon signals. Table 4 shows the proton and carbon assignments. The glycidol proton signals, five separate 1H signals, have very similar shifts as they had in the spectrum of p-methoxybenzyl glycidol ether (Figure 7). The multiplet at 3.1 ppm, the secondary epoxide proton, couples to two doublets of doublets at 2.6 and 2.8 ppm, the primary epoxide protons, and the two doublets of doublets at 3.4 and 3.7 ppm, the *sn*-3 protons. The protons next to the bromine (position 6') create a 2H triplet at 3.4 ppm that couples to the 2H pentet at 1.9 ppm (position 5'). The diastereotopic protons at position 1' are assigned to the 2H multiplet at 3.5 ppm, which couples to the 2H pentet at 1.6 ppm, (position 2'). The remaining four protons, positions 3' and 4', are assigned to the overlapping pentets at 1.4 ppm.

The next step was to link two bromohexyl glycidol ethers through a naphthalene diol (Figure 18). In this Williamson ether synthesis, NaH was used to deprotonate the phenolic alcohols on the 2,7-dihydroxynaphthalene (**11**), which then, through an S_N2 attack on the primary alkyl bromides, form ether linkages with the hexyl glycidol ethers. An excess of the bromohexyl glycidol ether was used for this reaction. Addition of the naphthalene ring makes the subsequent compounds in this synthetic series easy to detect under UV light.

A comparison of the ^{1}H NMR spectra of **10** and **12** (Figure 19) shows the progression from bromohexyl glycidol ether (**10**, Figure 19a) to 2,7-di-(hexyl glycidol ether) naphthalene (**12**, Figure 19 b). There are two major differences in these spectra.

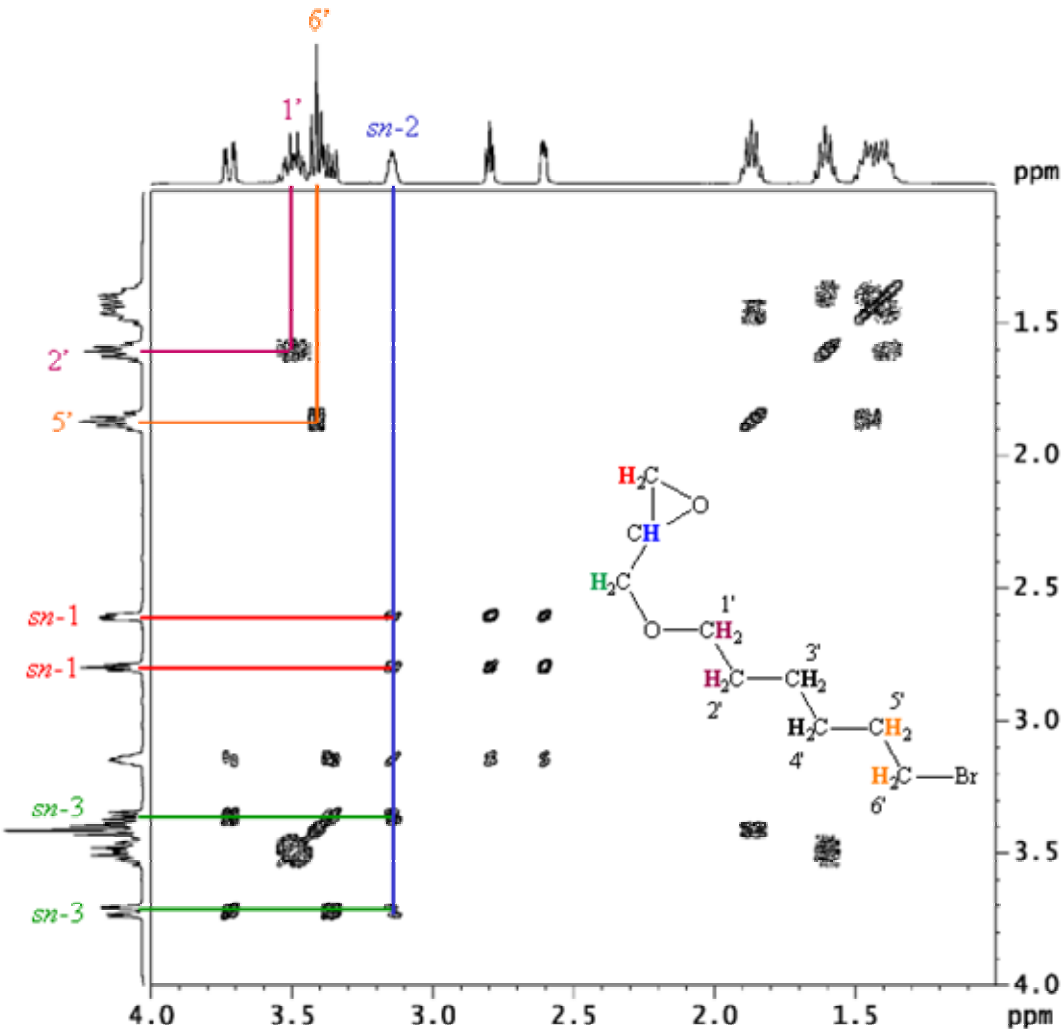


Figure 17: $^{1}H^{1}H$ COSY of **10**.

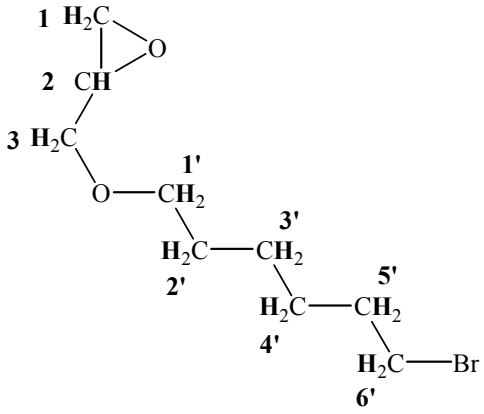


Table 4: NMR data for **10** in $CDCl_3$

	^{1}H δ (ppm)	Integration	Multiplicity	J (Hz)	^{13}C δ (ppm)
1	2.79	1H	dd	4.6, 2.8	44.1
	2.6	1H	dd	5.0, 2.7	-
2	3.14	1H	m	-	50.7
3	3.36	1H	dd	11.5, 5.9	71.3
	3.73	1H	dd	11.5, 2.9	-
1'	3.5	2H	m	-	71.2
2'	1.6	2H	p	7.1	29.3
3'	1.4	2H	m	-	27.8
4'	1.4	2H	m	-	25.1
5'	1.86	2H	p	7	32.5
6'	3.46	2H	t	6.8	33.8

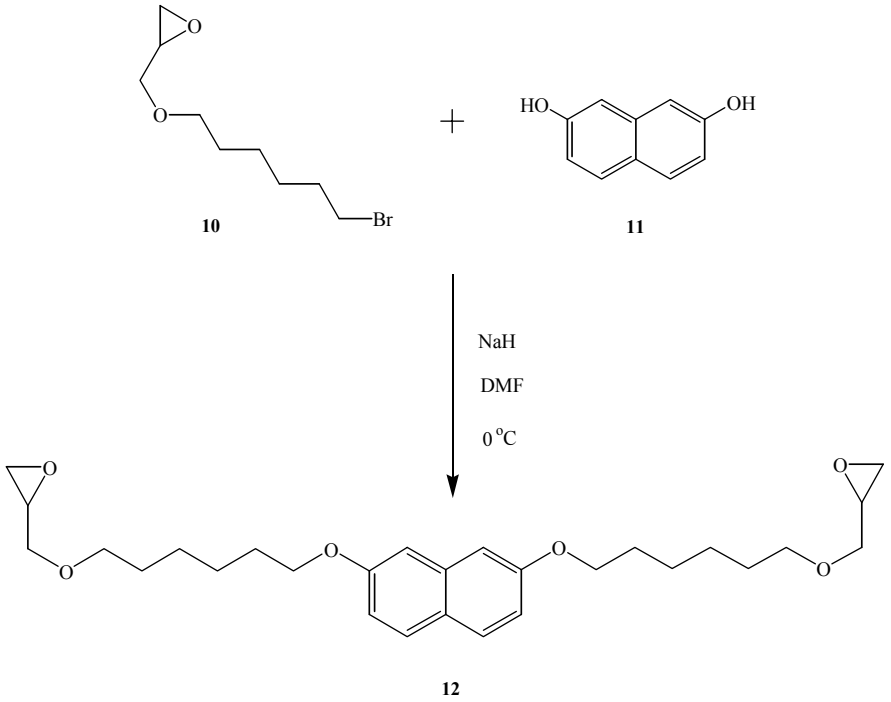
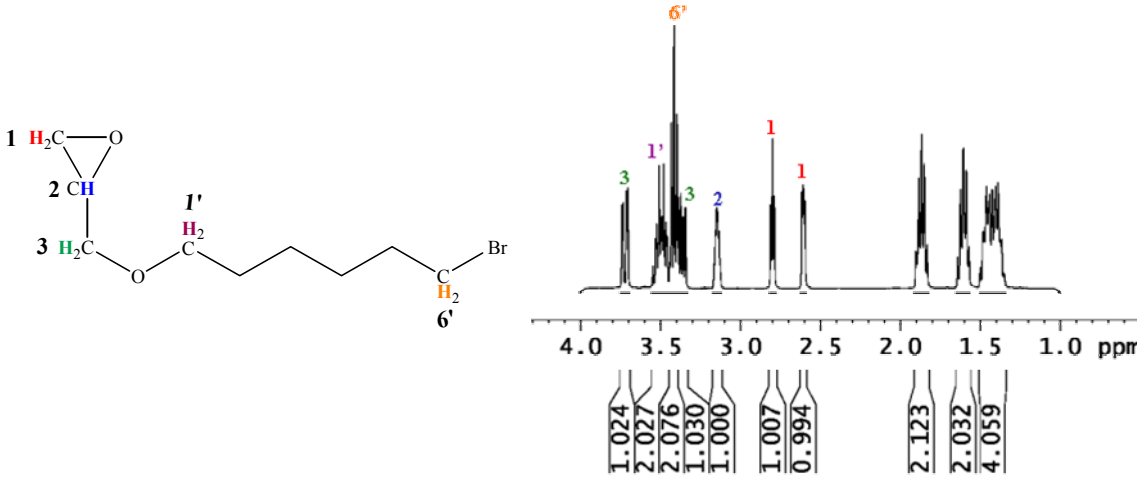
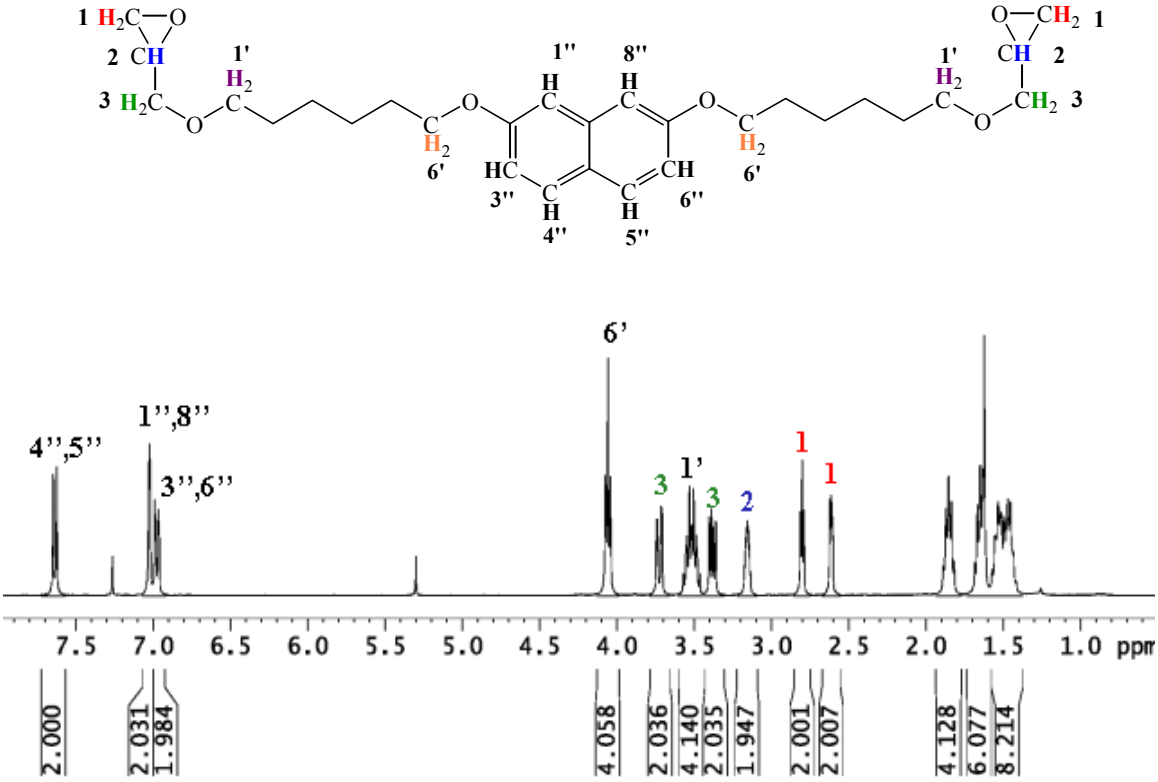


Figure 18: Synthesis of **12**.



a.



b.

Figure 19: ^{1}H NMR of **10** (a) and **12** (b).

First, integration in the spectrum of **12** shows that for every naphthalene moiety (six aromatic protons), there are two glycidols (total integration of 10H for the glycidol proton signals), accounting for one disubstituted naphthalene ring to two glycidol moieties. The second major difference is that the triplet corresponding to position 6' of the hexyl chain shifts from 3.4 to 4.1 ppm, upon conversion of the alkyl halide to the ether. Carbon assignments were determined using HMQC and are shown in Table 5.

The next step in this synthesis is to open the epoxides (Figure 20). It is reported that allyl alcohol (**13**) in the presence of sodium methoxide can be used to open at the epoxide and serve as a protecting group that can be removed in the presence of ethers. [8] The synthesis of **14** from **12** is shown by the ^{1}H NMR spectra (Figure 21). There are two major differences in the spectra that show the epoxides have been opened and substituted at the *sn*-1 positions with the allyl group. First, there are six olefinic protons in the spectrum of compound **14** (Figure 21b.), a 2H multiplet at 5.9 ppm corresponding to the two 2''' CH's, and two 2H doublet of doublets at 5.2 ppm, accounting for the four terminal double bond protons (3'''). Also, there is a 2H doublet at 2.5 ppm that results from the two *sn*-2 alcohol protons, similar to the *sn*-2 alcohol proton signals observed in previous spectra that are in other figures. In addition, when the epoxide is opened with the allyl group at *sn*-3, the glycidol protons and protons alpha to ethers (1' and 6') overlap, resulting in only two signals (3.5 and 4.0 ppm), as confirmed by COSY and HMQC. Assignments of all ^{1}H and ^{13}C signals are shown in Table 6.

The *sn*-2 carbon chain is added by another nucleophilic substitution reaction using NaH and 1-iododecane (**15**) to yield compound **16** (Figure 22). The yield for this reaction was relatively low (24%), possibly due to poor solubility of the starting materials

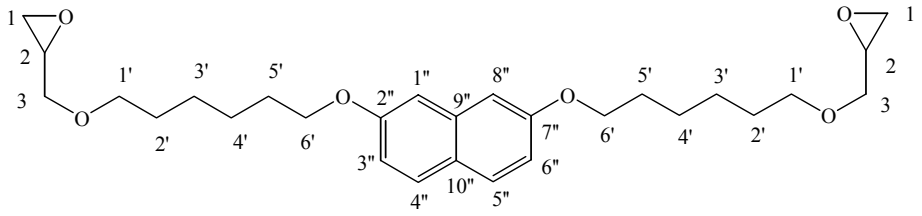


Table 5: NMR data for **12** obtained in $CDCl_3$.

	^{1}H δ (ppm)	Integration	Multiplicity	J (Hz)	^{13}C δ (ppm)
1	2.61	2H	m	-	44.31
	2.8	2H	m	-	
2	3.16	2H	m	-	50.9
3	3.39	2H	m	-	71.51
	3.72	2H	dd	3.0, 11.5	
1'	3.51	4H	m	-	71.48
2'	1.65	4H	p	7.2	29.62
3'	1.47*	4H	m	-	25.95
4'	1.47*	4H	m	-	25.88
5'	1.85	4H	p	6.8	29.19
6'	4.06	4H	t	6.5	67.76
1", 8"	7.02	2H	s	-	105.93
2", 7"	-	-	-	-	157.57
3", 6"	6.97	2H	dd	2.2, 8.8	116.2
4", 5"	7.63	2H	d	8.8	129.01
9"	-	-	-	-	135.91
10"	-	-	-	-	127.09

*Overlapping signals: 3' and 4'.

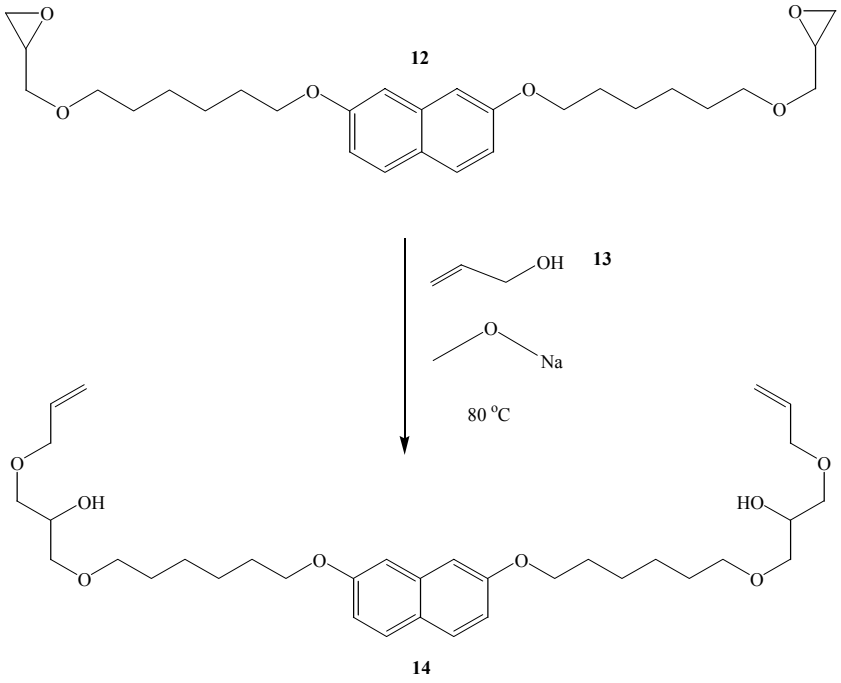
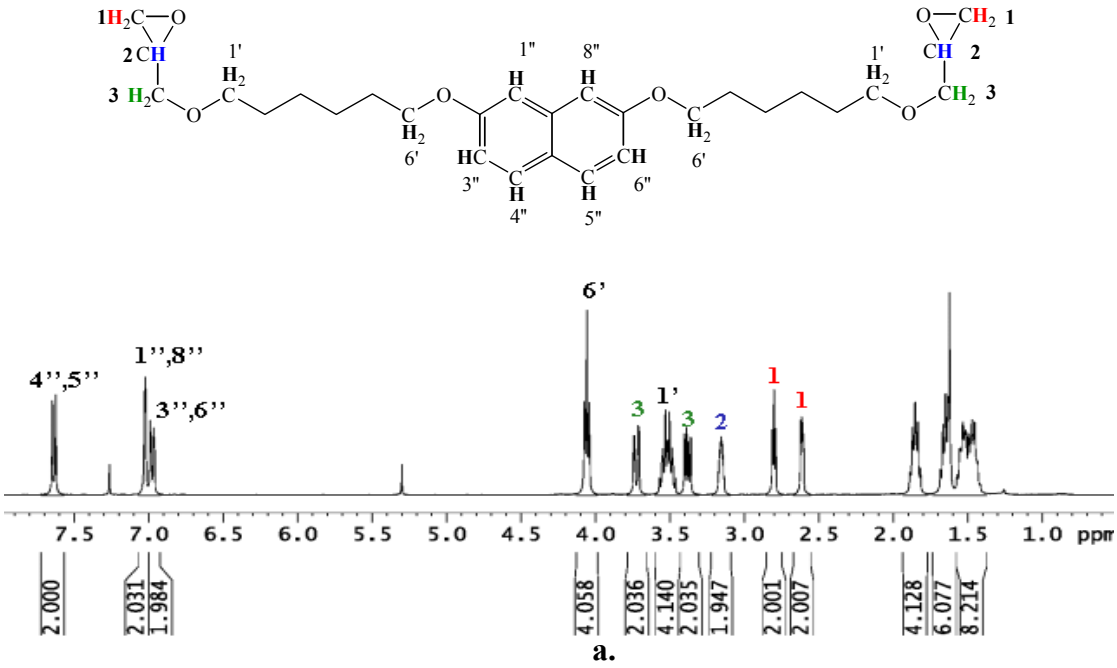
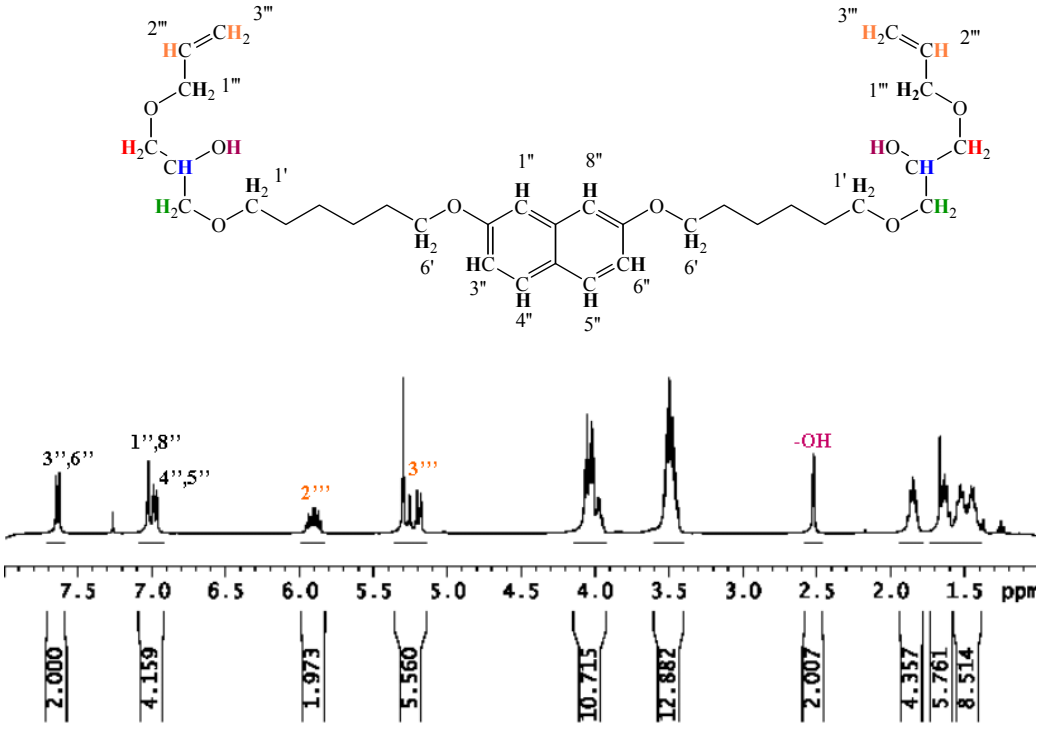


Figure 20: Synthesis of **14**.



a.



b.

Figure 21: ^{1}H NMR of **12** (a) and **14** (b).

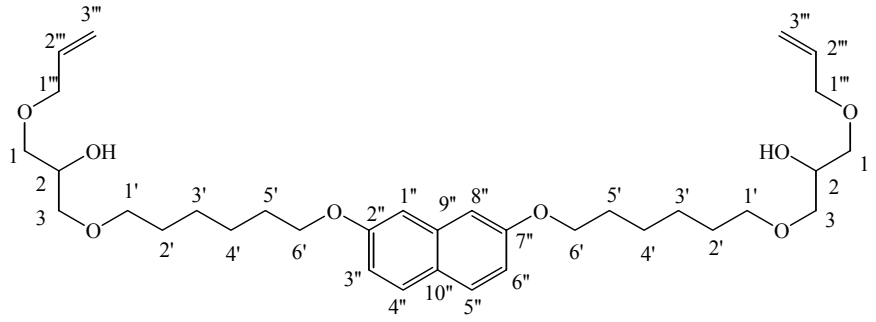


Table 6: NMR data for **14** obtained in $CDCl_3$.

	^{1}H δ (ppm)	Integration	Multiplicity	J (Hz)	^{13}C δ (ppm)
1	3.50*	4H	m	-	**
2	3.98	2H	m	-	69.5
3	3.50*	4H	m	-	**
2-OH	2.5	2H	d	4.1	-
1'	3.50*	4H	m	-	**
2'	1.6	4H	p	7.1	29.5
3'	1.4	4H	m	-	25.9
4'	1.5	4H	m	-	25.96
5'	1.8	4H	p	7	29.2
6'	4.00*	4H	m	-	67.8
1", 8"	7	2H	d	2.1	105.9
2", 7"	-	-	-	-	157.6
3", 6"	6.9	2H	dd	2.3, 8.8	116.2
4", 5"	7.6	2H	d	8.8	129
9"	-	-	-	-	135.9
10"	-	-	-	-	124.1
1'"	4.00*	4H	m	-	72.3
2'"	5.8	2H	m	-	134.5
3'"	5.2	4H	m	-	117.2

*Overlapping signals: 1, 3, and 1'; 1''' and 6'

** Carbon signals ranging from 71.3 ppm – 72.3 ppm: 71.3, 71.8, 72.3

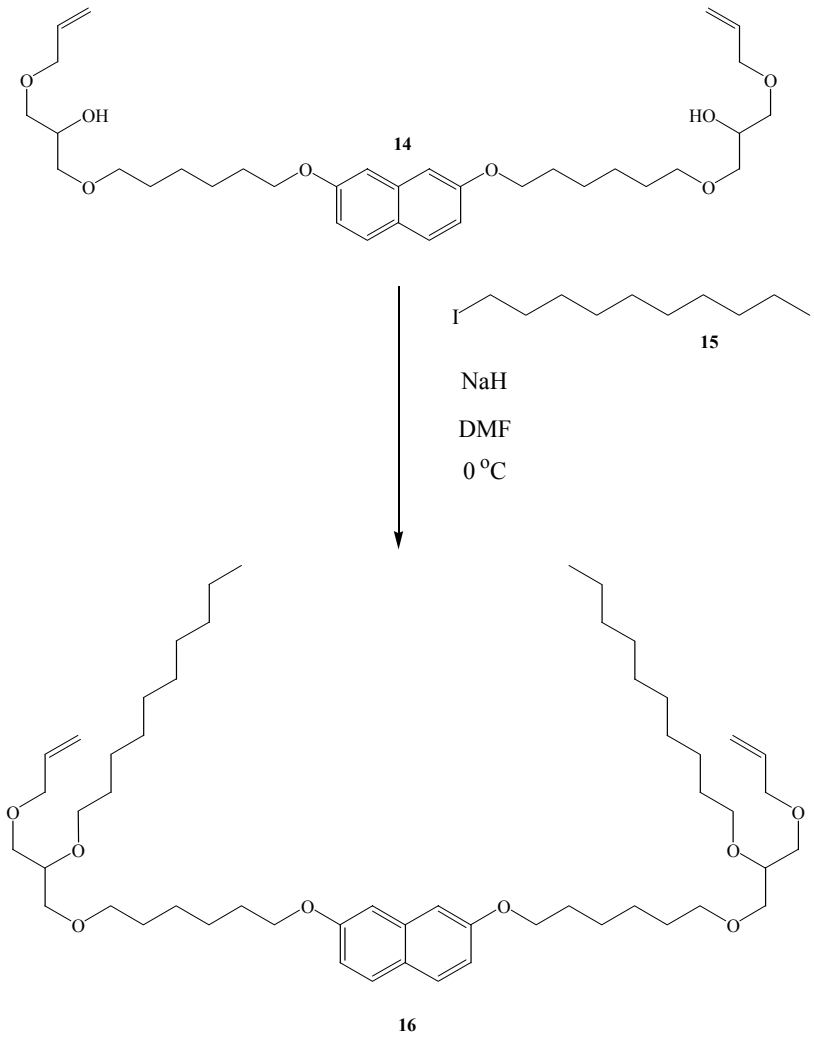


Figure 22: Synthesis of **16**.

in DMF. ^{1}H NMR was used to confirm the synthesis of **16** from **14** (Figure 23). All ^{1}H and ^{13}C signal assignments, confirmed by $^{1}H^{1}H$ COSY and HMQC, are shown in Table 7. The 2H doublet at 2.5 ppm seen in the spectrum for **14** (the *sn*-2 –OH's) is gone in the ^{1}H NMR for **16.** Additionally, there is a 6H triplet at 0.9 ppm that represents the two terminal methyl groups of the *sn*-2 decane chains. The other new proton signals from the *sn*-2 chains overlap with signals that were already present in the previous spectrum; however, the integration values account for the addition of both decane chains.

Next, the sn-3 positions are deprotected (Figure 24). The allyl group is removed using Wilkinson's catalyst, $C_{54}H_{45}ClPRh$ to give **17**.[8] The progression from **16** to **17** is confirmed by ^{1}H NMR (Figure 25). In the spectrum of **17** (Figure 25b), the removal of the allyl group is proven by the loss of the six olefinic protons, 5.0 to 6.0 ppm. The 2H triplet at 1.8 ppm is the result of two *sn*-1 alcohols. A 1.5 x 10^{-6} M solution of **17** in MeOH compound was found to have excitation and emission maxima of 234 nm and 344 nm respectively. If the lipid probe was being made to study interactions with a specific peptide, a different fluorophore (with similar chemistry to the dihydroxynaphthalene) could be used to provide the necessary fluorescence properties.

The final step in this synthesis was to add the desired phosphate group to the *sn*-3 positions of **17** (Figure 26). This was attempted under two sets of reaction conditions.[13.14] The first conditions were unsuccessful.[13] The second conditions, described in this paper, gave the desired product (**20**).[14] The addition of the phosphatidyl choline occurs through two steps. First, ethylene chlorophosphate (**18**) was added to the primary alcohol positions through an S_N2 reaction. Second, trimethylamine (**19**) was used to open up the ethylene phosphate ring to give a phosphatidylcholine (**20**).

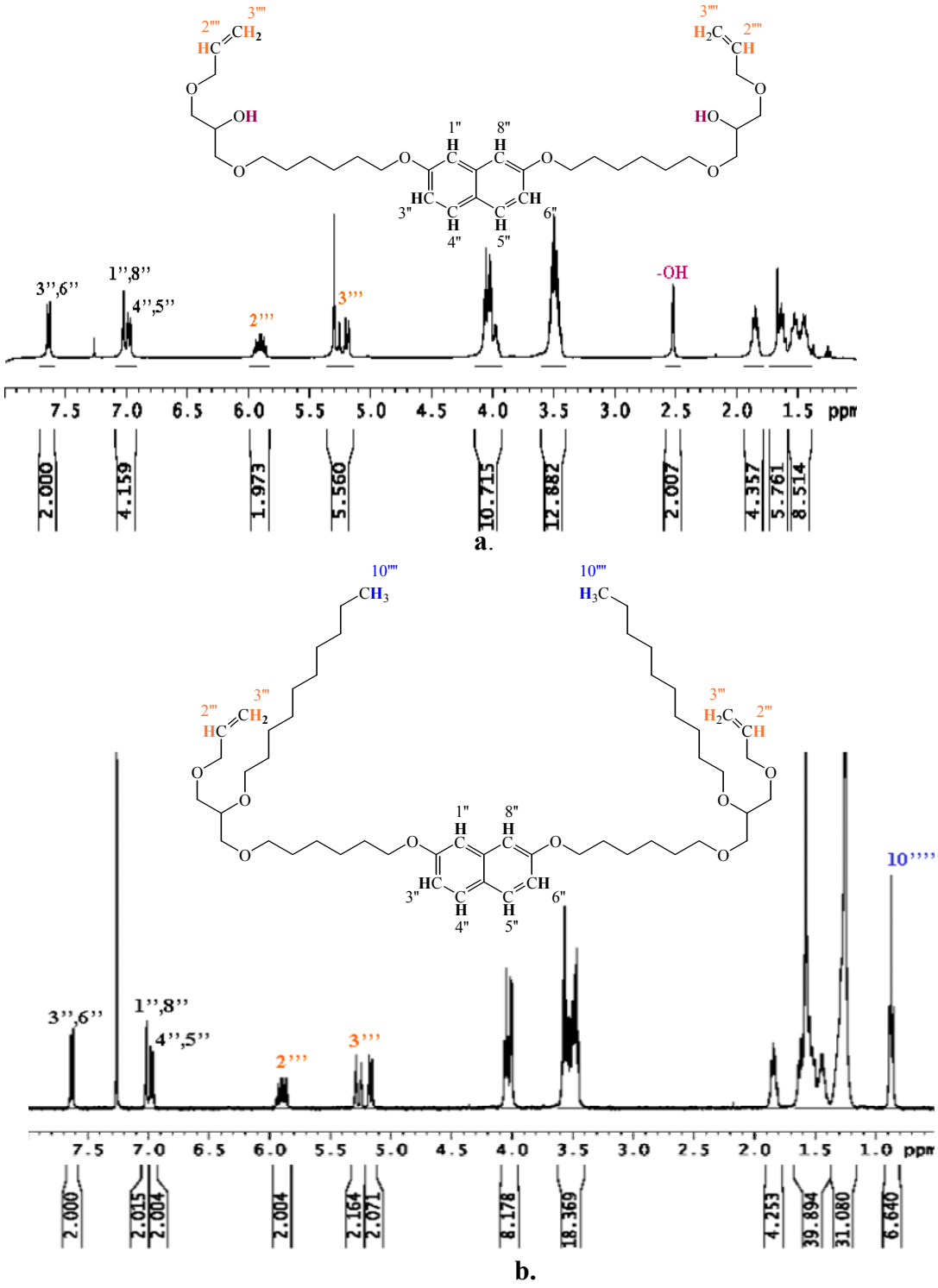


Figure 23: ^{1}H NMR of **14** (a) and **16** (b).

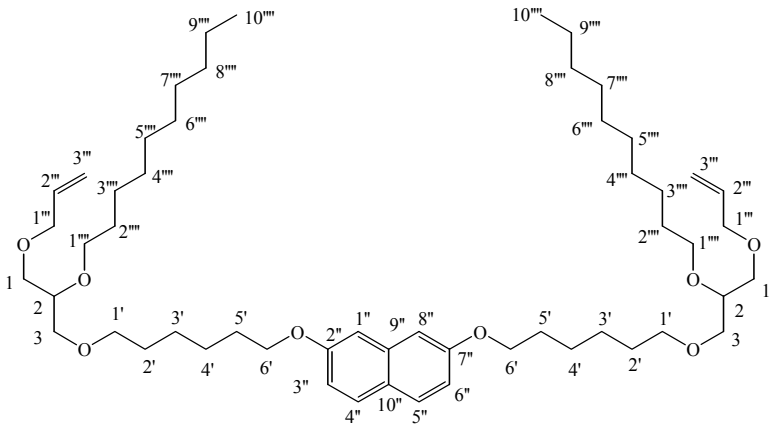


Table 7: NMR data for **16** obtained in $CDCl_3$.

	^{1}H δ (ppm)	Integration	Multiplicity	J (Hz)	^{13}C δ (ppm)
1	3.50*	4H	m	-	**
2	3.50*	2H	m	-	**
3	3.50*	4H	m	-	**
1'	3.50*	4H	m	-	**
2'	1.50*	4H	m	-	***
3'	1.50*	4H	m	-	***
4'	1.50*	4H	m	-	***
5'	1.85	4H	p	6.8	29.2
6'	4.05	4H	t	6.5	63
1", 8"	7.02	2H	d	2.2	105.9
2", 7"	-	-	-	-	157.5
3", 6"	6.97	2H	dd	2.3, 8.8	116.1
4", 5"	7.63	2H	d	8.9	129
9"	-	-	-	-	135.9
10"	-	-	-	-	124.1
1'''	4	4H	dd	1.2, 5.5	71.4
2'''	5.9	2H	m	-	134.8
3'''	5.25	4H	m	-	116.8
1''''	3.50*	18H	m	-	65-80
2''''	1.50*	18H	m	-	***
3''''-9''''	1.3	35H	m	-	***
10''''	0.88	3H	t	7.03	14.1

*Overlapping signals: 1, 2, 3, 1', and 1'''''; 2', 3', 4', and 2''''

**Carbon signals ranging from 67.8 ppm – 77.8 ppm: 67.8, 70.1, 70.6, 70.7, 72.3, 77.8

*** Carbon signals ranging from 22.6 ppm – 31.8 ppm: 22.6, 25.7, 25.9, 25.9, 26.0, 29.3, 29.4, 29.4, 29.5, 29.6, 30.0, 31.8

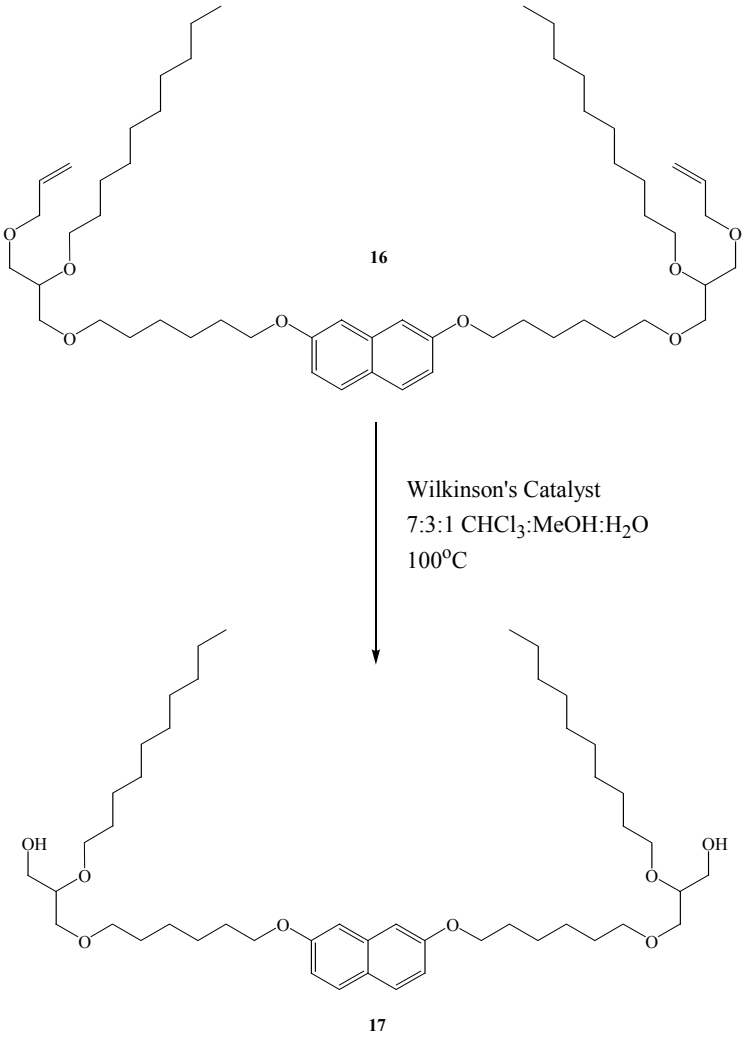
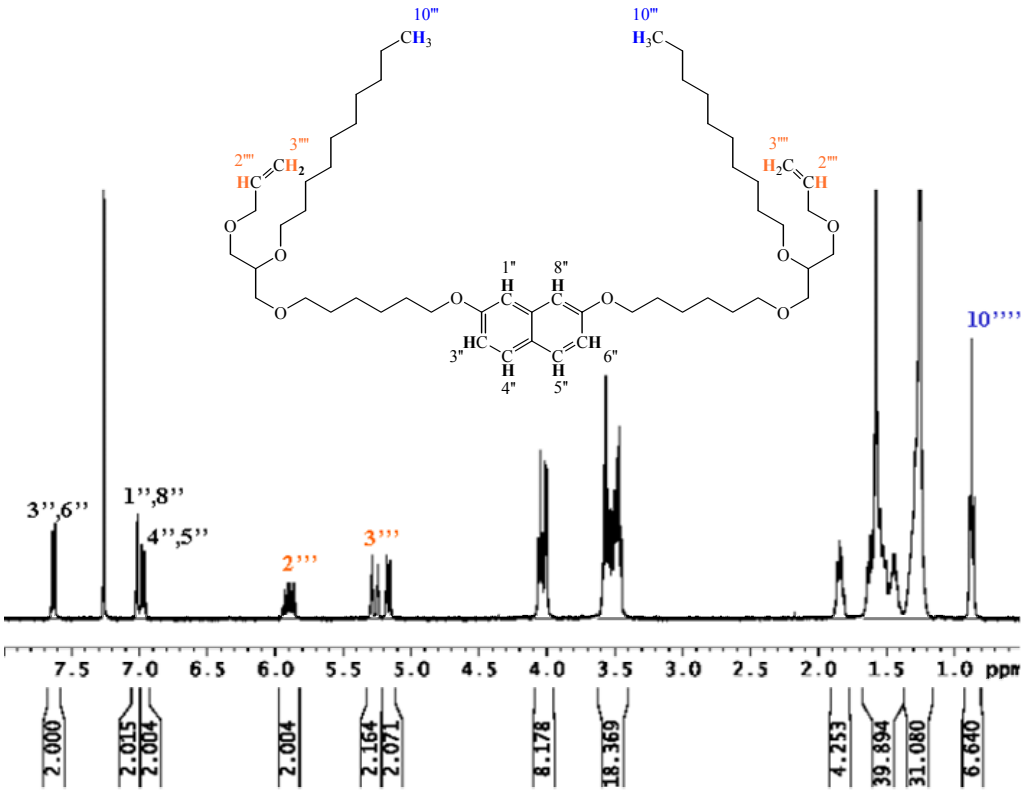
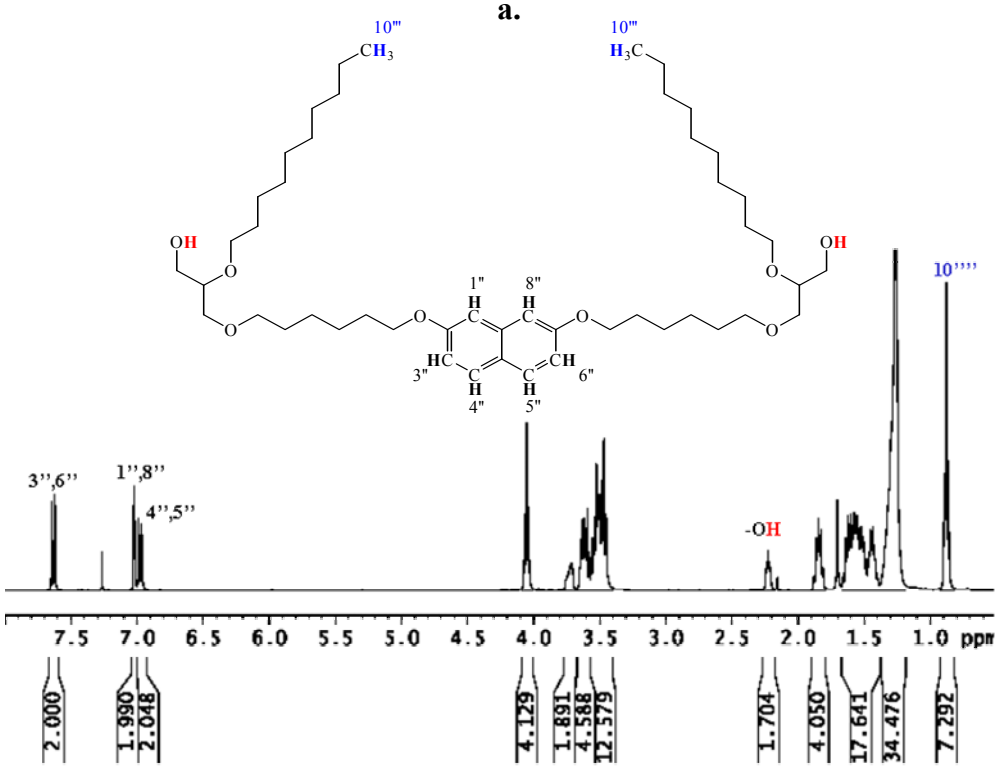


Figure 24: Synthesis of **17**.



a.



b.

Figure 25: ^{1}H NMR of **16** (a) and **17** (b).

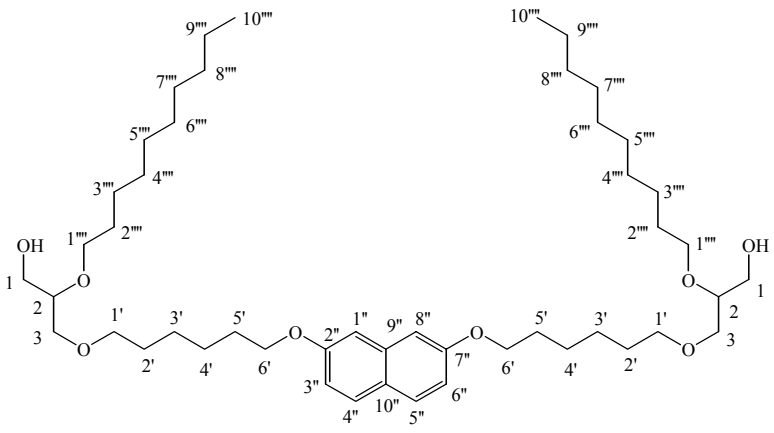


Table 8: NMR data for **17** obtained in $CDCl_3$.

	^{1}H δ (ppm)	Integration	Multiplicity	J (Hz)	^{13}C δ (ppm)
1	3.7	2H	m	-	**
1	3.62*	2H	m	-	-
2	3.62*	2H	m	-	**
3	3.50*	4H	m	-	**
1-OH	2.25	2H	t	-	-
1'	3.50*	4H	m	-	**
2'	1.58*	4H	m	-	***
3'	1.58*	4H	m	-	***
4'	1.58*	4H	m	-	***
5'	1.85	4H	p	-	29.2
6'	4.05	4H	t	6.5	67.8
1", 8"	7.02	2H	d	2.2	106
2", 7"	-	-	-	-	157.6
3", 6''	6.97	2H	dd	2.4, 8.9	116.2
4", 5"	7.63	2H	d	8.9	129
9"	-	-	-	-	135.9
10"	-	-	-	-	124.1
1""	3.50*	4H	m	-	**
2""	1.58*	4H	m	-	***
3""-9""	1.27	34H	m	-	***
10""	0.88	6H	t	6.6	14.1

*Overlapping signals: 1 and 2; 3, 1', and 1''''; 2', 3', 4', and 2''''

**Carbon signals ranging from 66.0 ppm – 78.2 ppm: 66.0, 70.4, 70.9, 71.7, 78.2

***Carbon signals ranging from 67.8 ppm – 77.8 ppm: 22.7, 25.9, 26.0, 26.1, 29.2, 29.3, 29.4, 29.6, 29.6, 30.0, 31.9

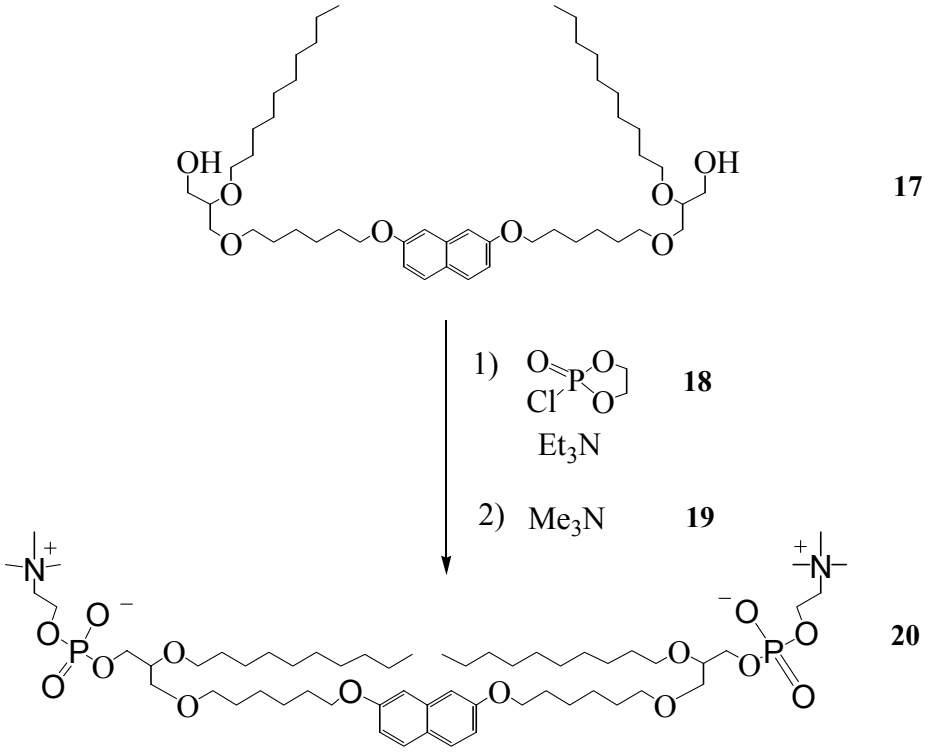


Figure 26: Synthesis of **20**.

The yield for this reaction was only 15%; however, conditions could be optimized in the future.

The structure of **20** was confirmed by ^{1}H NMR, ^{13}C NMR, $^{1}H^{1}H$ COSY, HMQC, and ^{31}P NMR. ^{1}H and ^{13}C NMR data is tabulated in Table 9. Substitution of the *sn*-3 alcohol is confirmed through a comparison of the ^{1}H NMR spectra of **17** (Figure 27a) and **20** (Figure 27b). Specifically, the *sn*-3 alcohol protons appear as 2H triplet at 2.25 ppm in Figure 26a, but are not present in the spectrum of **20**. Also, the spectrum of **20** (Figure 27b) shows a new 18H singlet at 3.30 ppm that correspond to the six methyl groups of the phosphatidylcholines. Positions 1, 1''', and 2''' were assigned to signals at 4.27, 3.90, and 3.60 ppm respectively. These are characteristic chemical shift values for similar protons in standard phosphatidylcholines. The remaining signals were assigned and confirmed using $^{1}H^{1}H$ COSY and HMQC. ^{31}P NMR of **20** is shown in Figure 28. Phosphatidylcholines are reported to give a phosphorous signal slightly below 0 ppm (-0.25 ppm).[13] Initially, the ^{31}P spectrum of **20** was obtained in CD_3OD (Figure 28a). This spectrum shows a low, broad signal at 0 ppm. Another ^{31}P NMR spectrum (Figure 28b) was obtained by making a solution of **20** in CD_3OD and $CDCl_3$ and adding 0.2 M EDTA, resulting in two layers.[15] This spectrum shows one major signal at 3 ppm and some minor signals; this sample was 90% pure by integration of this spectrum. The excitation and emission maxima for a 3.75×10^{-4} M solution of this compound in methanol were 325 nm and 344 nm respectively.

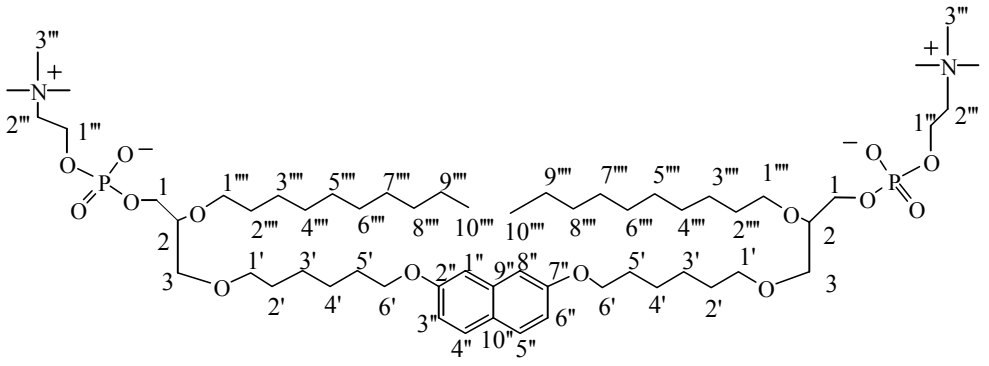


Table 9: NMR data for **20** obtained in CD_3OD

	1H δ (ppm)	Integration	Multiplicity	J (Hz)	^{13}C δ (ppm)
1	4.27	4H	m	-	61.3
2	3.60*	2H	m	-	68.4
3	3.60*	2H	m	-	72.5
	3.50*	2H	m	-	
1'	3.50*	4H	m	-	73.4
2'	1.53*	4H	m	-	**
3'	1.53*	4H	m	-	**
4'	1.53*	4H	m	-	**
5'	1.85	4H	p	6.3	32.1
6'	4.07	4H	t	6.3	69.8
1", 8"	7.1	2H	s	-	108
2", 7"	-	-	-	-	159.9
3", 6"	6.92	2H	dd	2.2, 8.8	118.1
4", 5"	7.62	2H	d	8.9	130.9
9"	-	-	-	-	138.5
10"	-	-	-	-	126.6
1'''	3.60*	4H	m	-	80.2
2'''	3.9	4H	m	-	67.2
3'''	3.2	18H	s	-	55.6
1''''	3.60*	4H	m	-	80.4
2''''	1.53*	4H	m	-	**
3'''' - 9''''	1.29	28 H	m	-	**
10''''	0.86	3H	t	6.5	15.4

Overlapping signals: 2, 3, 1''' and 1''''; 3 and 1'; 2', 3', 4', and 2''''

**Carbon signals ranging from 24.7 ppm - 34.0 ppm: 24.7, 28.0, 28.2, 31.3, 31.4, 31.6, 31.6, 31.7, 31.7, 34.0

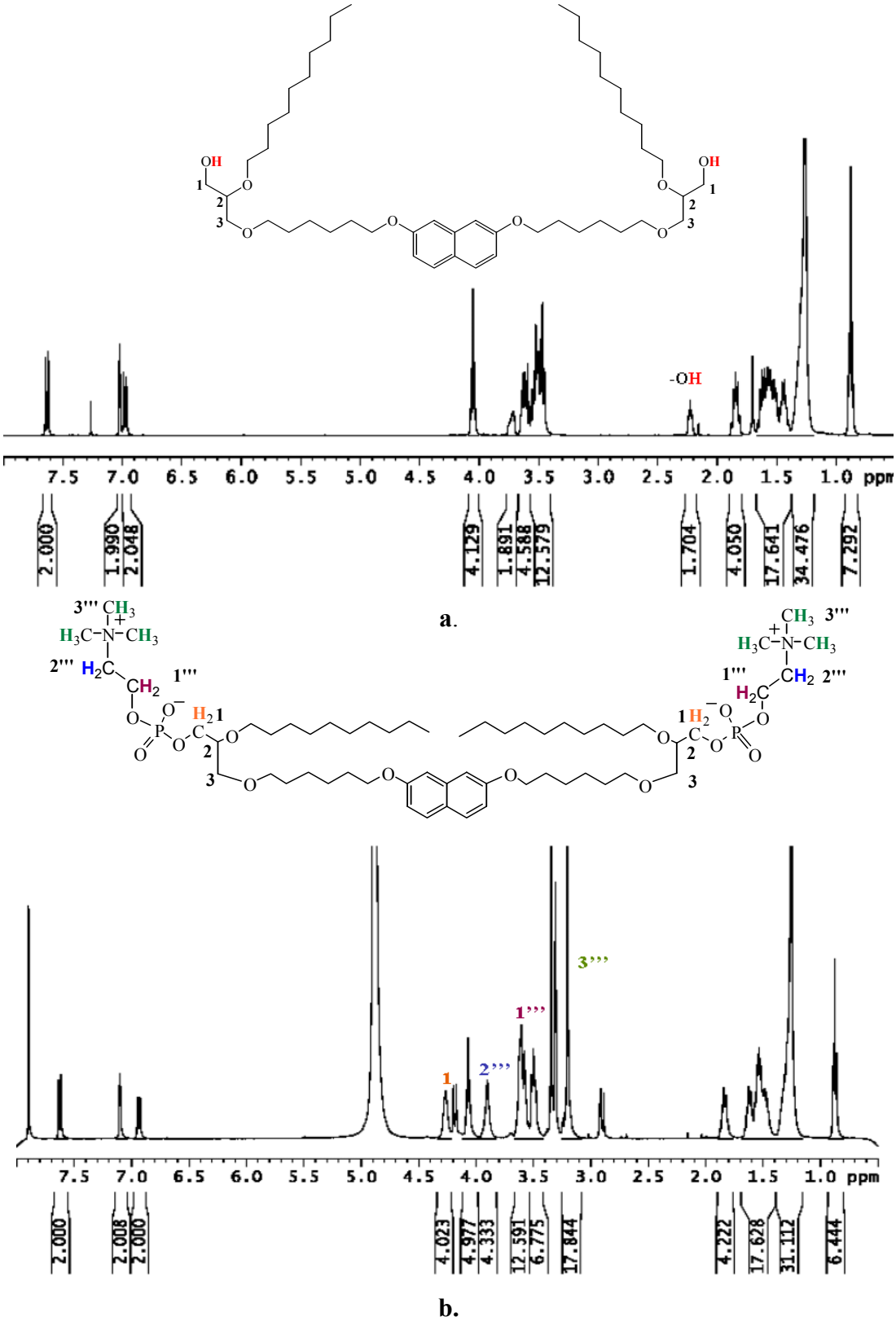


Figure 27: ^{1}H NMR of **17** (a) and **20** (b).

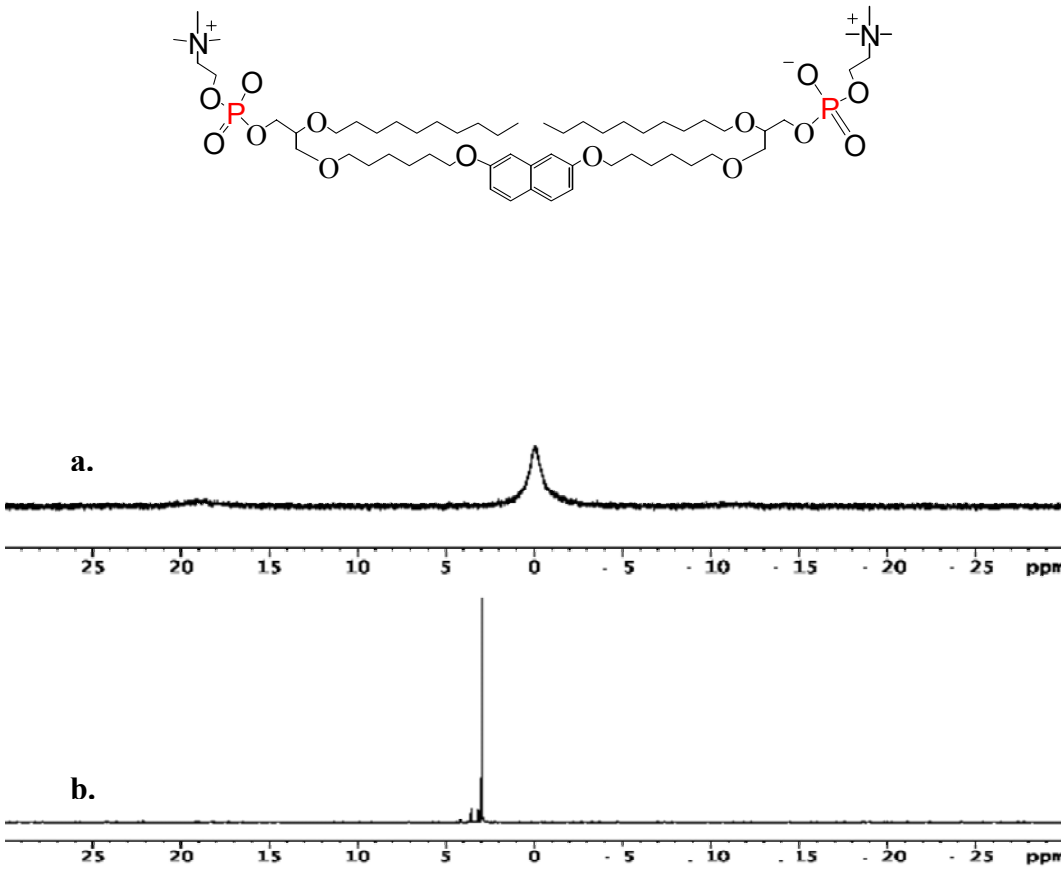


Figure 28: ^{31}P NMR of **20** in CD_3OD (a) and ^{31}P NMR of **20** in $CD_3OD/CDCl_3$ and 0.2 M EDTA in D_2O (b).

CONCLUSION

Because phospholipid structure plays such an important role in the structure and function of membranes, it is important to be able to elucidate lipid structures and also to study interactions within membranes. Two synthetic routes to phospholipid analogs were explored in this research. First, a synthetic pathway to UV-active diacylglycerides (DAGs) that have *sn*-1 and *sn*-2 ester-linked fatty acids and a chromophore at *sn*-3 was developed. Second, a synthetic pathway to a tethered phospholipid analog with a fluorophore anchored between the *sn*-1 alkyl chains was developed.

The first synthesis produced UV active diacylglyceride derivatives (Figure 2); these structures could be made with varying fatty acid composition to produce a series of standard DAG derivatives. Phospholipids derived from natural sources could be treated with phospholipase C followed by addition of the chromophore (the p-methoxy benzyl group) that was used in synthesis of DAG derivatives (Figure 2). The naturally derived lipids could then be compared to synthetic standards. However, it was seen in this work that the *sn*-2 fatty acid of *sn*-1,2 DAGs with an OH at *sn*-3 readily transesterify to the *sn*-3 position (Figure 14). It is possible that the *sn*-2 fatty acid of the naturally derived lipid would also transesterify after hydrolysis of the *sn*-3 phosphate group with phospholipase C. This would prevent derivatization of the lipid with the p-methoxybenzyl moiety at *sn*-3, thereby making it impossible to derivatize the lipids in order to compare to the synthetic DAG derivatives.

In the future, reaction conditions that limit the transesterification of fatty acids could be developed.[16] This would make it possible to remove the *sn*-3 protecting group of **7**, and replace it with the desired phosphate group to give **8**. Additionally, in order to use

the synthetic DAG derivatives developed in this work as standards to elucidate natural lipid structures, it will be necessary to develop conditions for derivatization of natural phospholipids.

The second synthesis was to prepare tethered lipid analogs that span the bilayer with a fluorophore held in the middle of the *sn*-1 alkyl chains (Figure 3). Ether linkages were used in this synthesis to avoid any problems associated with transesterification, as seen in the previous synthesis. The synthetic method developed in this work could be improved in the future by increasing yields of particular reactions. Specifically, addition of the alkyl chain to the *sn*-2 positions to give **16** (Figure 22) only gave 24% yield. This is probably due to poor solubility of the reactants in DMF. One option for improving yield could be changing the solvent. Additionally, yield may be improved by using a phase transfer catalyst similar to the synthesis of **5** (Figure 8) in which tetrabutylammonium bromide was used to prevent micelle formation. Additionally, the final step in this synthesis, addition of the phosphatidylcholine head group, gave 15% yield. This reaction, and purification of the final product, could also be optimized to increase yield. Other reactions in this synthesis had yields of 50 % or better.

In the future, the lipid probe (**20**) could be altered by changing alkyl chain lengths to accommodate a specific bilayer thickness and by changing the fluorophore. The dihydroxynaphthalene used for the development of a synthetic route to the lipid probe could be replaced in the future with a fluorophore that has chemical properties similar to the dihydroxynaphthalene, but appropriate fluorescence properties for its intended use.

EXPERIMENTAL

General. All solvents were purchased from Burdick & Jackson with the exception of DMF, which was purchased from Fisher Chemicals. Triethylamine, 2,7-dihydroxynaphthalene, tetrabutylammonium bromide, and oxalyl chloride were purchased from Acros. Diisopropylethylamine, allyl alcohol, sodium hydride, calcium hydride, and p-methoxybenzyl chloride were purchased from Aldrich. Stearic and oleic acids were from Fisher. Palmitic acid and glycidol (R and S) were purchased from Sigma. Wilkinson's catalyst ($C_{54}H_{45}ClPRh$) and 1-iododecane were purchased from Avacado. Silica gels were purchased from EM Science; silica gel 60 (0.040-0.063 mm, 230-400 mesh) was used for flash column chromatography (FCC), and silica gel 60 PF_{254} containing gypsum was to make plates for radial chromatography. Dichloromethane and triethylamine were both distilled over calcium hydride. DMF was distilled over molecular sieves.

Radial chromatography was performed using a model 8924 chromatatron from Harrison Research, and plates were prepared according to the Harrison Research chromatatron instruction manual. NMR experiments were performed using a Bruker 400 MHz Avance DRX spectrometer. ^{1}H NMR assignments were verified with $^{1}H^{1}H$ COSY, and ^{1}H ^{13}C HMQC experiments were done to assign ^{13}C signals based on the proton-carbon correlations. IR data was obtained using a Mattson Polaris FT-IR.

Synthesis of Glycerophospholipid Analogs

P-methoxybenzyl glycidol ether (3). Glycidol (300 μL, 4.50 mmol) was combined with p-methoxybenzyl chloride (674 μL, 4.95 mmol) in DMF (7 mL) and cooled to 0^{o} C. NaH (0.199 g 60% NaH, 4.95 mmol) was added and the reaction mixture

was stirred at 0° C under N_2 for approximately 3 hours. The reaction was quenched by the addition of water (10 mL), and the mixture was partitioned between H_2O and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 (3 x 15 mL). The combined organic layers were concentrated under reduced pressure and subjected to flash column chromatography using a solvent gradient ranging from 10% to 20 % EtOAc/hexane. Fractions containing the product were combined and concentrated to give **3** as a clear, colorless oil (0.568 g, 65%). NMR data is shown in Table 1. IR (film): 2998, 2934, 2910, 2858, 2837, 1612, 1514, 1465, 1302, 1249, 1091, 1034, 901, 821, 767, 582 cm^{-1}.

***Sn*-1 acyl *sn*-2 OH *sn*-3 p-methoxybenzyl ether (5a-b).** Palmitic acid (**4a**, 0.382 g, 1.49 mmol) was combined with **3** (0.289 g, 1.49 mmol), N,N-diisopropylethylamine (571 μL, 3.28 mmol), and tetrabutylammonium bromide (1.057 g, 3.28 mmol) in DMF (10 mL).[5] The reaction mixture was stirred under reflux at approximately 110° C for 30 hours. The mixture was partitioned between H_2O and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 (3 x 15 mL). The combined organic layers were concentrated and subjected to FCC using a solvent gradient of 2% to 10% diethyl ether/hexane. Fractions containing **5a** were combined, concentrated and subjected to radial chromatography using a 2mm plate thickness and a solvent gradient of 2% to 5% diethyl ether/hexane. Fractions containing the product were concentrated to give **5a** as off white solid (0.3183g, 55%). This procedure was followed for the addition of stearic acid to **(3)** to form **5b**. NMR data for **5a** and **5b** is shown in table 2. IR (film) of **5a**: 3450, 2953, 2916, 2849, 1662 cm^{-1}. IR (film) of **5b**: 3444, 2916, 2850, 1740, 1613, 1515, 1464, 1180, 1253 cm^{-1}.

***Sn*-1 acyl *sn*-2 oleic *sn*-3 p-methoxybenzyl ether (7a-b).** Oleic acid (**6**, 111 μL, 0.350 mmol) was combined with oxalyl chloride (34.6 μL, 0.396 mmol) and catalytic DMF in dist. CH_2Cl_2 (10 mL). The reaction mixture was stirred under N_2 at room temperature for 1 hour. The mixture was concentrated under N_2 giving oleic acid chloride (**6b**) as a white solid. CH_2Cl_2 was added until the solid was completely dissolved. This acid chloride solution was added dropwise, through an addition funnel, into a flask containing **5b** (0.112 g, 0.233 mmol) and triethylamine (166 μL, 1.19 mmol) in dist. CH_2Cl_2 (5 mL) at 0^o C.[6] The mixture was stirred at 0 ^{o}C for 2 hour and was then partitioned while cold between 5% HCl (15 mL) and CH_2Cl_2 (15 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were concentrated and subjected to FCC using a solvent gradient of 2% to 10% diethyl ether/CH_2Cl_2. Fractions containing the product were combined and concentrated, giving **7b** (0.102 g, 59%). NMR data for **7a-b** is shown in table 3. IR (film) of **7b**: 3001, 2925, 2854, 1743, 1613, 1514, 1465, 1248, 1172, 1112, 1038, 821, 722 cm^{-1}. IR (film) of **7a**: 2924, 2853, 1742, 1612, 1514, 1467, 1248, 1171, 1099, 1038 cm^{-1}.

Removal of methoxybenzyl group with DDQ. DDQ (0.012 g, 0.0522 mmol) was added to **7a** (0.037 g, 0.0522 mmol) in 10% MeOH/CH_2Cl_2. The reaction was stirred for 48 hours.[11] Water (15 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 x 15 mL). The combined organic layers were concentrated and subjected to FCC using CH_2Cl_2. Removal of the methoxybenzyl group resulted in a mixture of products, primarily *sn*-1,3 diacylglyerides. The ^{1}H NMR spectrum for the major products is shown in Figure 14.

Removal of methoxybenzyl group with ceric ammonium nitrate. Ceric ammonium nitrate (2.5 g in 35 mL deionized H_2O) and montmorellonite (2.5 g) were combined and concentrated under reduced pressure for approximately 2 hours. **7b** (0.017 g, .0233 mmol) was combined with ammonium nitrate/clay (0.03g) in CH_2Cl_2. The solvent was removed by concentrating under reduced pressure. The mixture was placed in the microwave for 2.6 minutes in a sealed tube.[12] The mixture was then extracted with 15% ether/CH_2Cl_2, and then concentrated. The mixture was then subjected to radial chromatography using a 2mm plate and a solvent gradient of CH_2Cl_2 to 15% ether/CH_2Cl_2. Removal of the methoxybenzyl group resulted in a mixture of primarily *sn*-1,3 diacylglyerides. The ^{1}H NMR spectrum for the major products is shown in Figure 14.

Synthesis of Lipid Probes

Bromohexyl glycidol ether (10). Glycidol (0.60 ml, 8.99 mmol) was combined with 1,6-dibromohexane (**9**, 3.05 ml, 19.8 mmol) in 10 ml distilled DMF and cooled to 0 ^oC. NaH (0.3954 g, 9.89 mmol) was added slowly. The mixture was capped and stirred overnight, allowing it to come to room temperature. The mixture was partitioned between H_2O and ether. The aqueous layer was extracted with ether (3 x 15 mL). The combined ether layers were concentrated and subjected to FCC using a solvent gradient of 10% to 50% EtOAc/hexane. Fractions containing only the desired product were combined to give **10** (1.07 g, 50%) as a colorless oil. NMR data for **10** is given in Table 4. IR (film): 2999, 2912, 2858, 2837, 1613, 1514, 1465, 1302, 1249, 1174, 1091, 1038, 901, 820, 767, 578 cm^{-1}.

2,7-Di-(hexylglycidol ether) naphthalene (12). A solution of **10** (0.8503 g, 3.587 mmol) in DMF (15 mL) was combined with 2,7-dihydroxynaphthalene (**11**, 0.2298 g, 1.435 mmol) and cooled to 0 ^{o}C. NaH (0.115 g, 2.87 mmol) was added slowly. The mixture was capped and stirred overnight, allowing it to come to room temperature. The mixture was partitioned between H_2O and ether. The aqueous layer was extracted with ether (3 x 15 mL). The combined ether layers were concentrated and subjected to FCC using a solvent gradient of 2% to 10% MeOH/CH_2Cl_2. Fractions containing only the desired product were combined and concentrated to give **12** (0.443 g, 65%) as a white solid. NMR data for **12** is given in table 5. IR (film): 3056, 2998, 2937, 2861, 1630, 1515, 1388, 1254, 1210, 1160, 1106, 834 cm^{-1}.

1-Allyloxy-3-(6-{7-[6-(3-allyloxy-2-hydroxy-propoxy)-hexyloxy]-naphthalen-2-yloxy}-hexyloxy)-propan-2-ol (14). A solution of **12** (0.3905 g, 0.743 mmol) in 18 mL of 5.59 x 10^{-5} M sodium methoxide in allyl alcohol (**13**) was stirred under reflux at 80^{o}C for 24 hours.[8] The mixture was partitioned between H_2O and CH_2Cl_2, and the aqueous layer was extracted with CH_2Cl_2 (3 x 15 mL). The combined organic layers were concentrated and further purified by radial chromatography using a 2 mm plate thickness and a solvent gradient of 1% MeOH/CH_2Cl_2 to 10% MeOH/ CH_2Cl_2. The fractions containing pure **14** were combined and concentrated under reduced pressure to yield **14** (0.3904g, 89.3%) as a pale yellow oil. NMR data for 10 is given in table 6. IR (film): 3425, 2915, 2849, 1632, 1515, 1459, 1388, 1260, 1209, 1113 cm^{-1}.

2,7-Bis-[6-(3-allyloxy-2-decyloxy-propoxy)-hexyloxy]-naphthalene (16). A solution of **14** (0.0870 g, 0.148 mmol) in 12 ml DMF was cooled to 0^{o}C in an ice bath. 1-iododecane (**15**, 0.190 ml, 0.887 mmol) and 60% NaH (0.0136 g, 0.340 mmol NaH) were

added. The reaction was capped, stirred, and allowed to come to room temperature overnight. The mixture was then partitioned between H_2O and CH_2Cl_2, and the aqueous layer was extracted with CH_2Cl_2 (3 x 15 mL). The combined organic layers were concentrated; after concentration, excess DMF was removed under reduced pressure (1-5 mmHg) with gentle heating. The mixture was then further purified by radial chromatography using a 2mm plate thickness and a solvent gradient of 1% MeOH/CH_2Cl_2 to 10% MeOH/CH_2Cl_2. The fractions containing pure **16** were combined and concentrated under reduced pressure to yield **10** (0.0312 g, 24.2%) as a colorless oil NMR data for **16** is shown in table 7. IR (film): 2926, 2849, 1637, 1520, 1449, 1389, 1270, 1220, 1123 cm^{-1}.

1-Hydroxy-3-(6-{7-[6-(3-hydroxy-2-decyloxy-propoxy)-hexyloxy]-naphthalen -2-yloxy}-hexyloxy)-propan-2-ol (17). Wilkinson's catalyst (0.013 g, 0.014 mmol) was added to **16** (0.077 g, 0.089 mmol) in 5 ml 7:3:1 EtOH:toluene: H_2O. The mixture was stirred under reflux at 100^oC for 48 hour and then partitioned between H_2O and CH_2Cl_2.[8] The aqueous layer was extracted with CH_2Cl_2 (3 x 15 mL), and the combined organic layers were further purified by radial chromatography using a 2 mm plate thickness and a solvent gradient of 3% MeOH/CH_2Cl_2 to 20% MeOH/ CH_2Cl_2. The fractions containing pure **17** were combined and concentrated under reduced pressure to yield **17** (0.049 g, 69.6%) as a colorless oil. NMR data for **17** is shown in table 8. Flourescence spectroscopy was used to determine the excitation and emission maxima, 234 nm and 344 nm respectively, using a 1.5 x 10^{-6} M solution of **17** in MeOH. IR (film): 3429, 2926, 2855, 1631, 1524, 1461, 1454, 1210, 1117 cm^{-1}.

Phosphoric acid mono-[2-decyloxy-3-(6-{7-[6-(2-decyloxy-3-phosphonooxy-propoxy)-hexyloxy]-naphthalen-2-yloxy}-hexyloxy)-propyl] ester (20). Triethylamine (0.086 mL, 0.439 mmol) was added to a solution of **17** (0.1732 g, 0.220 mmol) in dry benzene (5 mL). This was cooled to 0 ^{o}C and ethylene chlorophosphate (**18**) (0.050 mL, 0.527 mmol) was added dropwise. The mixture was stirred at 0 ^{o}C for five minutes and then at room temperature for two hours. This mixture was filtered to remove precipitate ($Et_3N{\cdot}HCl$) and concentrated under reduced pressure. The concentrated oil was immediately transferred to a pressure tube using dry acetonitrile (15 mL), and the solution was cooled in an acetonitrile dry ice bath. Trimethylamine (**19**) (2mL) was added; the tube was closed; and the mixture was stirred at 65 ^{o}C for 48 hours.[14] The mixture was concentrated under reduced pressure and subjected to radial chromatography using a solvent gradient of 20% MeOH/$CHCl_3$ – 100% MeOH. A UV active band that had an R_f less than 0.1 was scraped from the plate and the compound was extracted using 50:45:5 $CHCl_3$:MeOH:H_2O. This was concentrated under reduced pressure to give **20** (0.037 g, 15%) as a white solid. NMR data for **20** is shown in table 9. Flourescence spectroscopy was used to determine the excitation and emission maxima, 325 nm and 344 nm respectively, using a 3.75 x 10^{-4} M solution of **20** in MeOH. IR (film): 2925, 2854, 1631, 1457, 1247, 1212, 1094, 1049, 956, 925, 830 cm^{-1}.

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